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INTRA-ARTICULAR ANTIMICROBIAL THERAPY AND LAVAGE FOR THE MANAGEMENT OF ARTHRITIS IN CALVES

GANESH. G.

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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Ganesh. G

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

In calves, joint disease has many economic implications. The losses occur in the form of death or culling of affected and unrecovered animals, expensive treatment and poor production performance. According to Russel *et al.* (1982) joint disease is the second most important cause of lameness in cattle and among the joint diseases, arthritis predominates. Arthritis may be infectious and non-infectious or degenerative. On a comparative basis, frequency of occurrence of degenerative arthritis in ruminants is far less than infectious arthritis (Singh and Tayal, 1993).

Most commonly occurring type of infectious arthritis in ruminants, especially in young stock is 'Joint-ill', the synonyms of which include 'Joint-felon', 'Polyarthritis', 'Navel-ill' etc. It is characterized by abscess formation at the umbilicus, lesions in various joints, most frequently the stifle, hock and knee, and frequently abscesses in some of the internal organs. (Wooldridge, 1934). The microorganisms involved in infectious arthritis are *Corynebacterium pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp. and *Salmonella* spp. (Pratap *et al.*, 1977), *Mycoplasma bovis* and *Haemophilus somnus* as reported by Orsini (1996).

Symptoms may vary according to the degree of virulence of the organism. In a few cases joints have been found swollen at birth. In the ordinary case symptoms may occur in about a week or ten days after birth upto as long as six months or even longer. In advanced cases the calf will be reluctant to suckle and feed with severe loss of condition and the prognosis is not favourable. Complete recovery may occur in a number of cases, but in most recoveries the patients remain unthrifty for a long time (Wooldridge, 1934). Usually the physical symptoms are very characteristic, and it includes swollen and painful joints which are warm to touch. Lameness is often the most predominant symptom (Gustafson, 1993; Jackson, 1999 and Bennet, 2005).

A careful examination provides valuable aid in early diagnosis and prognosis. Analysis of blood and synovial fluid help in better understanding of the pathogenesis of the disease. A clear understanding of the pathogenesis of arthritis will ensure that affected animals are treated at the earliest possible stage, so that lameness does not become irreversible. Radiographic examination of affected joint can be used effectively to assess the chronicity of infection, but generally does not reveal anything other than joint effusion in early stages (Moulvi *et al.* 1993).

The aim of treatment of septic arthritis is to eliminate the infection completely, to restore intra-articular sterility and to remove harmful enzymatic and proteinaceous material in order to minimize damage to the articular cartilage. If treatment is instituted within five days of onset of the disease, the prognosis is better than otherwise (Goldenberg and Reed, 1985).

On screening the available literature, the treatment of arthritis by intra-articular antimicrobial therapy and lavage were found scanty. Hence the present study was undertaken. In this study the clinical, physiological, haematological, biochemical, radiographic and microbiological changes associated with arthritis in calves and the effectiveness of intra-articular antimicrobial therapy/ lavage with 10 per cent dimethyl sulfoxide solution for the management of arthritis were evaluated.

Review of Literature

2. REVIEW OF LITERATURE

2.1. Arthritis in calves

Wooldridge (1934) reported polyarthritis (joint-ill) as a disease occurring in young stock, due to infection by way of the umbilicus and occasionally before birth, characterized by abscess formation at the umbilicus and lesions in various joints, most frequently in the stifle, hock, and knee.

Van Pelt *et al.* (1966) classified infectious arthritis in calves with respect to its duration as acute, subacute or chronic and as primary, secondary or tertiary depending upon its mode of pathogenesis.

Russel *et al.* (1982) reported joint diseases, to be the second most important cause of lameness in cattle and septic arthritis, to be the most common cause of joint disease and suggested that joint diseases in cattle could be divided into non-inflammatory and inflammatory diseases. The non-inflammatory diseases included mainly osteochondrosis, degenerative joint disease, joint trauma, and haemarthrosis. The inflammatory diseases included non-infectious and infectious arthritis. Noninfectious arthritis included idiopathic arthritis, immune-mediated arthritis and synovitis caused by periarticular infections.

Prasanna (2003) recorded the incidence of suppurative arthritis of both knee joints in a calf which had the history of extra-abdominal umbilical infection.

2.2. Clinical signs of arthritis

Wooldridge (1934) reported that symptoms of polyarthritis in calves, foals and lambs varied according to the degree of virulence of the etiological organism. In the ordinary case, symptoms would occur in about a week or ten days after birth upto as long as six months or even longer. The symptoms were general depression, indisposition to suckle or feed, elevated body temperature by

2° to 4°F, stiffness of movement, irregular bowels, sometimes diarrhoeic, sometimes constipated and rapid loss of condition.

Van Pelt *et al.* (1968) stated that infectious arthritis of calves was attributable to metastasis of bacteria and pyrexia was a general manifestation.

According to Gustafson (1993) the infected joints of animals affected with septic arthritis were warm, swollen and painful. In light skinned animals, they may appear reddened secondary to increased local blood flow. The joint swelling was often periarticular and intracapsular. Initially the swelling consisted of edema and engorged blood vessels. Lameness was the predominant clinical sign in animals affected with septic arthritis.

Jackson (1999) reported lameness involving one or more limbs in calves affected with septic arthritis and the carpus, hock and stifle joints were the most commonly affected ones. Affected joints were distended, warm to touch and with painful movement. Pyrexia of upto 40°C would be present. The calf would be depressed and reluctant to get up and feed. In advanced cases, joint mobility was progressively reduced and it was immobile in ankylosed cases.

Bennet (2005) reported lameness as an important clinical feature in arthritis. Only about one third of infective arthropathies had systemic signs. Bacterial infections were generally with an acute onset. A more chronic, insidious onset was also possible, the so-called low-grade infections. The joints were usually swollen and painful on manipulation and warm to touch.

2.3. Diagnosis of arthritis

2.3.1. Haematological changes in arthritis

Pratap *et al.* (1977) conducted studies regarding haematological aspects of experimentally induced septic arthritis in buffalo calves. Haematological studies were made two days before inoculating the organisms intra-articularly and also

on the third and seventh days post-inoculation. The mean haemoglobin (Hb), packed cell volume (PCV) values of the experiment animals in the pre and post inoculative periods observed in this study were 10.83g and 35.08 per cent and 9.73g and 31.03 per cent respectively. These values were insignificant, although, there had been definitive decrease in their mean values in post-inoculative period. From the studies it was concluded that haematological examination was diagnostically insignificant in the early stages of septic arthritis.

2.3.2. Synovial fluid changes in arthritis

Warren *et al.* (1935) opined that, cells in synovial fluid were not to be considered as a true reflection of the cellular constituents in peripheral blood.

West *et al.* (1963) opined that the increased alkaline phosphatase activity in synovial fluid might be due to release of enzymes from leucocytes, pathological changes in synovial membrane or production and release of increased amount of enzymes by altered synovial tissues as the indicative of inflammatory changes in the joint.

According to Van Pelt and Langham (1968), increased volume of synovial effusion from infected joints was a constant finding, varying from one joint to another and relative to individual joint size. Yellow or amber synovial effusions were indicative of high bilirubin content and varied in direct proportion to the respective colour. It was also reported that infectious synovial effusions clotted rapidly in the absence of added anticoagulant.

Van Pelt (1974) reported poor to very poor mucin clot in case of septic arthritis and normal to fair in case of non-infectious arthritis.

Van Pelt (1975) opined that opacity and flocculation in synovial fluid were due to the presence of cartilaginous fragments and fibrils.

Coffman (1980) reported the estimation of glucose level in synovial fluid and blood/serum and comparison of the two as a meaningful diagnostic tool. It was also reported that the difference between them would be more in the presence of sepsis.

Bowman *et al.* (1983) reported significant increase in alkaline phosphatase in arthritis (162.75 ± 3.30 as compared to 132.75 ± 3.33 IU/L, the normal level) might be due to disruption of lysosomal activity that occurred within the joint.

According to Goldenberg and Reed (1985) any purulent effusion should be considered septic. It was also reported that a significant drop of synovial fluid sugar in septic arthritis might be due to the presence of more cells in the fluid to use glucose at a greater rate.

According to Lloyd *et al.* (1988b) blood contamination of the synovial fluid collected via arthrocentesis and the associated synovial response of the joint could be a source of error in interpretation of results.

Chawla *et al.* (1989a) studied the changes in biochemical constituents of synovial fluid in calves after inducing septic arthritis and reported that the synovial fluid became turbid, yellow and opaque with varying amounts of flocculent materials. The total protein content of the synovial fluid increased significantly on the third post-induction day and remained significantly high up to 45^{th} post-induction day.

Madison *et al.* (1991) opined cytologic evaluation of synovial fluid samples as the probable single most useful test in evaluating a joint with suspected infection and recommended to consider infection, when nucleated cell counts in the synovial fluid exceed 10,000 cells/microlitre.

Gustafson (1993) reported synovial fluid analysis as the most valuable parameter used to establish the diagnosis of septic arthritis. It was opined that

estimation of total protein or total solids, total nucleated cell count, and cytological examination in synovial fluid analysis would frequently give strong indications of sepsis.

Moulvi *et al.* (1993) opined the determination of mucin clot quality as a good index of presence of infection in the joint. Mucin precipitated by 2.5 per cent of glacial acetic acid contains all hyaluronic acid and about 75 per cent of proteins present in a normal synovial sample. It was also reported that identification of organism by Gram's staining of concentrated sediment of centrifuged synovial fluid provided rapid definitive diagnosis of septic arthritis.

According to Jackson (1999) the synovial fluid containing more than 60gram of protein per litre, more than 10,000 cells per microlitre and polymorph proportion greater than 90 per cent was suggestive of septic arthritis.

Rhode *et al.* (2000) considered cytological analysis of synovial fluid as the most important ancillary test for differentiating between infectious and non-infectious joint diseases.

Moulvi *et al.* (2001) reported that synovial glucose decreased significantly by day 21 after inducing infectious arthritis of carpal joint by intra-articular inoculation of *Stapyhlococcus aureus*.

May (2005) reported that the gross changes seen in the synovial fluid in association with bacterial infective arthritis were increased volume, turbid or blood tinged fluid, poor viscosity and clotting on exposure to air due to high fibrinogen content. The cytological changes typically seen in synovial fluid smears were markedly increased numbers of white blood cells, mostly neutrophils (more than five to ten neutrophils per high power field) and toxic neutrophils with pyknotic nuclei or ruptured or degenerated neutrophils.

2.3.3. Culturing of synovial fluid

Verschooten *et al.* (1974) reported difficulty in isolating organisms from cases of chronic arthritis. It was opined that culture and sensitivity results could not always be correlated with clinical responses to treatment.

Madison *et al.* (1991) recommended the treatment of inflammed joints presuming to be infected and treated as such, even if bacteria could not be isolated. It was also reported that culturing of synovial fluid yielded bacterial growth more often than did culturing of synovial membrane.

Gustafson (1993) reported two basic methods for recovering bacterial isolates: inoculation of blood agar plates and inoculation into brain-heart infusion broth blood culture bottles. Direct inoculation of synovial fluid onto blood agar plates resulted in earlier growth and led to lighter rates of isolation than sending transport swabs long distances to a microbiology laboratory.

Orsini (1996) reported *Streptococcus spp.* and *Escherichia coli* as the most commonly isolated organisms in young calves, followed by *Actinomyces pyogenes*, *Salmonella* spp., *Mycoplasma bovis*, and *Haemophilus somnus*.

Clements *et al.* (2005) reported that a negative result from a bacterial culture of synovial fluid from clinically infected or experimentally infected joints would lend support to the assertion that a positive bacterial culture of synovial fluid might not be a necessity for the diagnosis of bacterial infective arthritis.

May (2005) failed to yield a positive result in cases of bacterial infective arthritis by direct culture of infected synovial fluid. Success rates reported in both naturally occurring and experimental cases ranged from approximately 50 to 80 per cent .It was reported that the prior use of antibacterial agents, even as a single dose would reduce the chances of a successful culture from synovial fluid even further.

2.3.4. Radiographic changes in arthritis

According to Jani (1987) plain radiography might not correlate with the extent of joint damage during the earlier stage of arthritis in calves.

Chawla *et al.* (1989b) reported that venography and arteriography could be used effectively in studying the various consecutive changes produced in and around the joints following infectious arthritis. In animals with poor response to treatment tuft of venous channels were visualized proximal to the affected joint through venography and generalized hypervascularity and tortous vessels could be detected at areas proximal and distal to the affected joint in arteriography.

Jackson (1999) reported that in early stages of septic arthritis, soft tissue swelling around the joint might be the only abnormality visible on radiography. Radiographic evidence of advanced joint damage suggested a guarded prognosis for the case.

According to Verschooten *et al.* (2000) the clinical diagnosis of bone infection was straightforward. A swollen joint in a lame bovine could almost immediately be associated with a septic arthritis or infection close to a growth plate. Radiographs allowed a distinction between a primary subchondral and joint infection. Radiographic studies revealed that arthritis probably originated along the joint capsule, because direct infection via the epiphysis was not evident. It was also reported that joint became infected subsequent to subchondral bone infection and the swelling of the soft tissues induced periosteal reaction at the metaphysis.

Bist *et al.* (2001) conducted studies on contrast arthrography of the radiocarpal joint in calves. Double contrast technique was done using one to three millilitres of undiluted Urographin along with 15 to 25 millilitre air. Radiographs taken within three minutes of the injection of the contrast medium were of good quality. The articular structures were better visualized on using lower concentration (38 per cent) of the contrast material. Optimum dose of the positive

contrast agent for a radiocarpal joint in calf was reported to be three to four millitre. It was concluded that double contrast technique had no advantage over the positive contrast arthrography except delineation of joint capsule thickness.

Kumar and Agarwal (2001) reported that radiographs of buffalo calves with induced traumatic arthritis on day fifteen showed a marked soft tissue swelling around the joint capsule. Distension of the joint capsule was also marked without considerable change in joint space. No bony abnormalities were detected in the radiographs.

Moulvi *et al.* (2001) reported that radiographs taken on day 21 after inducing infectious arthritis of carpal joint in calves by intra-articular inoculation of *Stapyhlococcus aureus* showed considerable increase in soft tissue density and slight increase in joint space. These changes did not show much improvement even by 40^{th} day.

Desrochers (2004) opined that in calves with septic arthritis radiographic lesions had revealed a tendency to be more lytic, with less new bone formation in comparison to older animals.

According to May (2005) radiologically visible bony changes in septic arthritis could develop by one to two weeks after the onset of infection, and often progress rapidly to produce a marked periarticular periosteal bone reaction. Occasionally there was also mineralization of the periarticular soft tissues, particularly in more chronic cases. Subchondral bone erosions and irregular areas of subchondral bone sclerosis were the common radiographic features.

Ramanathan (2007) observed radiographic lesions such as widened joint space, intra-articular gas shadow, subchondral osteolysis and blurring of normal bone outline in calves affected with septic arthritis.

2.4. Intra-articular changes in arthritis

Bertone *et al.* (1987b) reported that release of cellular enzymes, plasmin and prostaglandins following joint sepsis led to depletion of proteoglycans (<5 days) from the cartilage matrix, alteration in cartilage pliability and subsequent collagen breakdown from increased vulnerability to mechanical forces.

According to May (2005), the degradation of cartilage in septic arthritis might be due to the dissolution of the glycosaminoglycans matrix, with subsequent collagen breakdown compounded by mechanical forces on the degraded matrix. In the later stages, gross changes appeared in the articular cartilage due to the damage of collagen fibres. It was also reported that the severity of cartilage destruction resulted from bacterial invasion of a joint consequent to the virulence of the infecting organism and the nature of the host's immune responses.

Madison *et al.* (1991) reported that histologic examination of synovial membrane rarely provided sufficient data to make a definitive diagnosis of various arthropathies with an exception of finding bacterial colonies in cases of infectious arthritis or finding urate crystals in cases of gout.

2.5. Complications of arthritis

Van Pelt *et al.* (1966) reported that lesions encountered in subacute to chronic joint infections due to *Corynebacterium pyogenes* were almost pathgnomonic and were characterized by extensive erosion and, in some portions of the joint, by severe destruction of the articular cartilages. This destruction was attributed to a combination of pannus formation and subchondral fibrosis. Pannus growth over opposing articular cartilages and the subsequent adhesion of such pannus growths if given time would obliterate the joint cavity.

Angus (1991) reported the development of granulation tissue (pannus) across the articular surface as a complication of acute septic arthritis. The lytic

action of the pannus on the collagen together with collagenase from neutrophils caused widespread erosion of articular surfaces. Finally organization of the inflammed tissues together with development of bony spicules (osteophytes) in the joints greatly diminished mobility and resulted in permanent stiffness. It was also observed that the swollen joints of lambs and sheeps affected with suppurative polyarthritis at necropsy contained upto 20 ml of greyish pus, congested and velvety synovia and erosion or ulceration in articular cartilages. Suppurative foci could be found in the kidneys, myocardium, lungs or meninges.

Desrochers (2004) recorded ankylosis of the joint, muscle atrophy and tendon deformities in calves with chronic septic arthritis as the post treatment complications even after control of infection.

2.6. Prognosis

According to Wooldridge (1934) prognosis of polyarthritis in affected animals was not very favourable, as the condition would be fatal in about 50 per cent of cases. Death was reported in two or three days or two or three weeks, but in most recovered animals unthriftiness was noticed for a long time. In other cases the joints remained permanently enlarged caused chronic lameness.

Radostits *et al.* (2003) reported that the prognosis in case of advanced septic arthritis was poor. Neglected animal might die or had to be destroyed because of open joints or pressure sores. Chronic arthritis and subsequent ankylosis greatly impeded locomotion and interfered with usefulness of the animal.

2.7. Treatment of arthritis

According to Fox (1983) dimethyl sulfoxide (DMSO) prevented the depolymerisation of hyaluronic acid by oxygen derived free radicals and through direct suppression of prostaglandin production.

Alsup and DeBowes (1984) reported that the anti-inflammatory property of DMSO was based on its ability to scavenge free radicals and to suppress prostaglandin production. Using DMSO, local and central mechanisms of analgesia had also been reported.

Orsini (1984) treated infectious arthritis of radio-carpal joint in cattle by injection of antibiotics through a needle into the affected joint cavity after it had been lavaged. Arthroscopy had also been recommended to prevent the interference of pus and fibrin clots with the antibiotic.

Brown and Newton (1985) stated that the fundamental goals in the treatment of septic arthritis as: 1. To clean the joints in order to avoid articular cartilage destruction and adhesion formation. 2. To compress the joint in the immature patient to avoid vascular embarrassment to the epiphysis and 3. To administer adequate doses of antibiotics to eradicate the joint infection and prevent secondary spread.

Bertone *et al.* (1987a) compared various treatments for experimentally induced equine infectious arthritis and concluded that systemic administration of antibiotics resulted in clinical improvement of affected animals compared with control animals. Treatments with higher doses of antibiotics decreased the rate of bacteriologic isolation and with joint drainage resulted in less loss of articular cartilage glycosaminoglycans than joints treated with antibiotics once daily. Arthrotomy with lavage resulted in less synovitis and less fibrin formation than treatment by through-and-through lavage performed one to three times. Delayed healing, fibrosis, and excessive granulation tissue complicated healing of all arthrotomy incisions.

Bertone *et al.*(1987b) observed that significant differences were not found between joints lavaged with electrolyte solution versus povidone-iodine solution for synovial total protein concentration, WBC count, results of synovial fluid and membrane bacteriologic culture, synovial membrane inflammation, or articular

cartilage glycosaminoglycan concentration and concluded that use of 0.1 percent povidone-iodine did not have an advantage over use of balanced electrolyte solution as an irrigation solution in through-and–through joint lavage for the treatment of infectious arthritis in equines.

Lloyd *et al.* (1988a) reported that the mean half-life of gentamicin in the synovial fluid after intra-articular administration was two to eight times longer than that in the plasma after intravenous administration. It was further reported that the mean synovial fluid concentration of gentamicin remained well above the minimal inhibitory concentration of gentamicin for many bacterial pathogens for 24 hours after intra-articular administration.

Lloyd *et al.* (1988b) reported that the bactericidal activity of gentamicin is dependent on the pH of the extracellular environment in an acidic medium; it is substantially decreased against otherwise susceptible microorganisms.

Chawla *et al.* (1989b) reported that in calves with induced infectious arthritis where antibiotics were given by systemic and local intravenous routes, soft tissue swelling reduced considerably and infection was checked effectively. Local intravenous injection of antibiotics provided a better concentration of antibiotics in and around the affected joints to produce satisfactory results.

Van Huffel *et al.* (1989) reported that arthrotomy and lavage, either alone or preceded by arthroscopy was superior to the less invasive techniques of drainage and irrigation in resolving clinical signs of septic arthritis. However, this procedure might not be effective for the carpus, because the carpal articulations are complex and extension into the bones and adjacent soft tissues is common.

Welch *et al.* (1989) evaluated the short-term effect of intra-articular injection of a 40 per cent concentration of DMSO and reported that it was no more irritating than injections of equal amounts of lactated Ringer's solution.

Adair *et al.* (1991) conducted experimental joint lavage in clinically normal horses to compare the effects of DMSO and buffered lactated Ringer's solution on intra-articular structures with that of arthrocentesis alone and reported that joint lavage induced significantly greater inflammatory reaction than arthrocentesis alone. But there was no significant difference in intra-articular inflammatory change induced by buffered lactated Ringer's solution, 10, 20 and 30 per cent DMSO. It was concluded that as DMSO has antimicrobial, hyaluronate protective and free radical scavenging properties, it might be advantageous for the treatment of septic and non-septic arthritis.

As stated by Angus (1991) the use of intra-articular corticosteroids was contra-indicated in lambs and sheeps affected with chronic suppurative arthritis, as it could exacerbate any erosion of the articular cartilages. In colostrum feeding lambs the use of non-steroidal anti-inflammatory agents had been recommended in the treatment of joint ill as against the use of corticosteroids, having immunosuppressant effect.

Ike *et al.* (1992) recommended intra-articular lavage in osteoarthritic joints of humans for the removal of debris and degradative enzymes and for the disruption of adhesion fibrins.

According to Gustafson (1993) the basic principles which existed in the treatment of septic arthritis were sterilization of the joint and prevention of bacterial and enzymatic joint destruction. It was opined that if sepsis could be identified early and treatment instituted, it could decrease bacterial numbers and minimize joint damage.

Tayal *et al.* (1994) reported DMSO to have a mild inflammatory reaction when injected into the normal bovine synovial joints. It was also reported that the transient mild inflammatory reaction caused by intra-articular administration of DMSO was less than that by lactated Ringer's solution. Hence DMSO was recommended for the treatment of many inflammatory diseases of the joint.

Hirsbrunner and Steiner (1998) used gentamicin-impregnated collagen sponges successfully in the treatment of chronic septic arthritis of radiocarpal joint in two cattle. Both animals were moderately to severely lame and refractory to systemic antibiotics, and one of them was refractory to joint lavage and local antibiotics. The infection was eliminated from both animals and they recovered without residual lameness.

Jackson (1999) recommended the use of an 'in and out' technique wherein fluid should be introduced into the joint through a single needle using a 20 or 50 ml syringe, if the 'through and through' lavage technique proved impossible.

Moulvi *et al.* (2002) reported that inflammation caused by daily intra-articular administration of gentamicin in the treatment of infectious arthritis in calves would be more severe than that caused by a single dose and would take a longer time to resolve. So single dose intra-articular administration of gentamicin was recommended than daily intra-articular administration of gentamicin.

According to Sharma *et al.* (2003) intra-articular transfusion of synovial fluid had therapeutic efficacy in acute aseptic arthritis in donkeys and synovia collected aseptically from the corresponding normal knee joint of the same animal was better than the one collected from the normal healthy joints of other donkeys.

May (2005) suggested, in the non availability of a pressurized delivery system, repeated flushing through the inlet portal with a high volume (20 or 50 millitre) syringe or by using a syringe technique with a single portal, alternately distending the joint with lavage fluid and then draining fluid back into the syringe.

2.8. Normal anatomy of knee joint in calves

2.8.1. Joint structures

Fournier *et al.* (1969) reported that the synovial membrane was freely permeable to electrolytes, sugars, some dyes and certain proteins but not to larger proteins or to certain antibiotics. The synovial membrane barrier was appreciably complicated by the rôle of mucin which, according to its degree of polymerization, would facilitate or hinder the flow of particles through the mesh like synovial membrane. The altered permeability of the synovial membrane might be secondary to a modification of capillary size, hydrostatic and osmotic pressures, or to altered hyaluronic acid polymerization. It was concluded that the synovial membrane could be considered as a delicate filter which was probably not as physiologically refined as kidney but was almost as complex and equally effective. It would be naïve to think of synovial membrane as a mesh with a varied and changing caliber; electrical and mechanical forces were also active.

According to McIlwraith (1981), the synovial membrane was a semipermeable membrane that controls the composition of the synovial fluid, a dialysate of plasma to which hyaluronate was added. Inflammation within the joint induced increased metabolic activity and vascular permeability of synovial membrane, resulting in synovial effusion and changes in synovial fluid composition.

Nickel *et al.* (1986) recorded the details regarding articular cartilage. According to the authors, the articular cartilage (*Cartilago articularis*) was bluish in the fresh state, smooth and it was attached to the bone by a narrow zone of calcified cartilage. Usually the cartilage was only a few millimeters thick. The cartilage accentuates the curvature of the articular surface. On concave articular surfaces it was thickest at the periphery and centrally on convex surfaces. The articular cartilage was traversed by a system of fine fibrils which were arranged in the direction of the greatest tension when the joint bears weight. Its elasticity gave a high degree of adaptability to the articular surfaces, which provided them

with the ability to absorb shocks. The delicate synovial layer was richly supplied by blood, lymph vessels and nerves and its inner surface was covered, often incompletely, by squamous cells derived from fibroblasts. It frequently formed synovial villi or folds (*plicae synoviales*) which contained fat cells and projected into the joint cavity. The synovial membrane secretes synovial fluid which acted as a joint lubricant.

Diarthrodial joints were composed of subchondral bone covered by hyaline articular cartilage, a fibrous joint capsule lined with synovium, and a joint cavity filled with fluid. Most diarthrodial joints were supported by ligaments, some had articular synovial fossae, and two joints (femorotibial and temporomandibular) contained menisci (Gustafson,1993).

Desrochers *et al.* (1997) reported that in cattle, the middle carpal joint and the carpometacarpal joint always communicated. The antebrachiocarpal joint communicated with the middle carpal joint between the ulnar and intermediate carpal bones. The middle carpal and carpometacarpal joints always communicated between the fourth and fused second and third carpal bones. It was concluded that individual variation of the carpus in cattle should be considered when diagnostic or treatment protocols were established.

Bist *et al.* (2001) carried out plain radiographic studies of carpal joint in calves. Accordingly it was reported that it consisted of three major joints namely, radiocarpal, intercarpal and carpometacarpal. Six carpals were arranged in two rows proximal and distal. The proximal row from medial to lateral consisted of radial, intermediate, ulnar and accessory carpal bones. In distal row, fused second, third and fourth carpal bones were present.

2.8.2. Synovial fluid

Van Pelt and Conner (1963a) studied the composition of synovial fluid from the normal bovine tarsus, proved to be relatively acellular. The mean

differential leukocyte values were: neutrophils- 6.0 ± 1.24%, lymphocytes- 49.08 ± 2.77%, monocytes- 38.22 ± 2.47%, macrophages- 5.93 ± 1.38% and eosinophils- 0.77 ± 0.22%. Basophils were never observed in stained smears.

Van Pelt and Conner (1963b) reported a synovial fluid sugar to blood sugar ratio of 1:1 in normal cattle.

According to Amrousi *et al.* (1966) normally the synovial fluid of bovines was a clear, colourless or straw coloured viscous, non-coagulable liquid with a normal mucin precipitate quality. The fluid was alkaline in reaction and with a lower specific gravity than serum. Compared to bovine serum, synovial fluid contained almost the same levels of sugar, urea, creatinine and phosphorus and was devoid of any traces of bilirubin. Young calves showed a significantly lower total leukocyte number, chloride level and sugar content and a higher calcium and in-organic phosphorus than bulls.

According to Singh and Tayal (1993) normal synovial fluid of cattle, buffaloes and camels was clear, colourless to yellow, viscid, free from blood and did not clot after standing. Cytologically the percentage of neutrophils was usually less than 10 per cent and macrophages were seen only occasionally.

Chauhan and Agarwal (2006) provided data regarding normal synovial fluid properties. Normally the synovial fluid was in small quantity in any joint and it was colourless or sometimes slightly yellow tinged. Normal synovial fluid was clear, transparent and odourless. Usually a three to four centimeter thread was formed in normal synovial fluid when a glass rod was dipped in the fluid and the length of thread was measured upon withdrawl of glass rod. The specific gravity ranged from 1.010 to 1.015 and normal synovial fluid did not clot upto two days under normal conditions at 4°C.

Materials and Methods

3. MATERIALS AND METHODS

3.1. SELECTION OF ANIMALS

The study was carried out in twelve calves affected with arthritis aged below six months and belonging to different breeds of either sex, presented to the College Veterinary Hospital at Mannuthy and University Veterinary Hospital at Kokkalai with the history of swelling at the knee joint and clinical signs suggestive of arthritis. Details about signalment and anamnesis were collected. All the calves were subjected to detailed clinical, physiological, haematological and serum biochemical evaluations. Radiographic and synovial fluid evaluations of the affected joint(s) were also carried out. Based on the nature of synovia, whether it was non-purulent or purulent, the calves were divided into Group I and II respectively consisting of six calves each. The calves in each group were serially numbered, *viz.*

Group I- I_1, I_2, I_3, I_4, I_5 and I_6

Group II- II_1, II_2, II_3, II_4, II_5 and II_6

3.2. PATIENT MANAGEMENT

3.2.1. Group I

Synovia was collected by arthrocentesis. It was performed by inserting a sterile hypodermic needle of 18 gauge size through the dorsolateral aspect of knee joint lateral to the tendon of extensor carpi radialis muscle under local infiltration anaesthesia with two per cent lignocaine hydrochloride[1]. Through the same needle used for arthrocentesis, gentamicin sulphate[2] at the dose rate of 4mg/kg body weight was infused into the joint cavity. A sterile dressing pad of cotton was placed around the knee joint and bandaged with an elastic bandage. The same procedure was repeated on the fifth day also in those calves which were not recovered by the initial therapy.

1. Xylocaine 2%- 30 ml vial, AstraZeneca Pharma India Limited, Bangalore.
2. Gentamicin injection- 1 ml equivalent to 40 mg, TTK Healthcare Limited, Chennai.

3.2.2. Group II

Through-and-through lavage technique was adopted for the treatment. Through a 18 gauge sterile hypodermic needle inserted as in Group I (ingress needle) (Fig.1.) dimethyl sulfoxide (DMSO)[3] 10 per cent solution was infused with pressure into the joint for irrigating the cavity and the outflow was maintained through another 18 gauge sterile hypodermic needle (egress needle) (Fig.2.) inserted between the distal dorsal rim of the radius and proximal dorsal articular rim of the radial carpal bone. After evacuating the fluid from the cavity, the egress needle was taken out and gentamicin at the dose rate of 4mg/kg body weight was infused into the joint cavity through the ingress needle and the needle was taken out. A sterile dressing pad was placed around the joint and bandaged with an elastic bandage as in Group I (Fig.3.). Same procedure was repeated on the fifth day also in animals that had persistent swelling.

To the calves of both the groups, gentamicin at the rate of 4mg/kg body weight was administered intramuscularly for five consecutive days from the first day of treatment onwards. Based on the result of culture and sensitivity test, for calf II_2 Sulphadiazine-trimethoprim[4] was administered intra-muscularly at the rate of one milliliter per 30 kg body weight for the five consecutive days.

Radiographic evaluation of the affected knee joint and collection of blood samples from the jugular vein for haematological and serum biochemical evaluation were carried out before treatment, on the fifth and fifteenth post treatment days.

3.3. MAIN ITEMS OF OBSERVATION

3.3.1. Signalment

Details regarding the animal such as age, breed, sex and joint(s) affected were recorded.

3.3.2. Anamnesis

History and duration of illness, previous treatment if any, previous illness/injury, details about colostrum intake, time taken for the calf to get up after birth and also history of deworming were recorded.

3.3.3. Physiological observations

Physiological parameters *viz.*, rectal temperature (°C), pulse rate (per minute) and respiratory rate (per minute) were recorded before treatment and then on the fifth and fifteenth post-treatment days.

3.3.4. Clinical observations

Subjective monitoring of the affected joint such as presence of joint swelling, pointing of limb whether present or not, ability to flex the limb, presence of warmthness and pain on palpation of the joint, presence of joint exudation and open joint were recorded (Fig.4, 5 and 6). Anderson and Desrochers (2004) lameness scoring system was followed for assessing lameness of each calf and it is as follows:

Scores/grades	Observations
0	Normal gait
1	Mild: walks easily, readily; bears full weight on foot and limb; but has an observable gait alteration; stands on all four limbs; line of back bone normal.
2	Moderate: reluctant to walk and bear weight but does use the limb to ambulate; short weight bearing phase of stride; rest the affected limb when standing; increased period of recumbency; arching of back bone.
3	Severe: reluctant to stand; refuses to walk without stimulus; non-weight bearing on affected limb; 'hoops' over the limb rather than bear weight; does not use limb when standing and lies down most of the time; back bone arched with caudo-ventral tip to pelvis.
4	Catastrophe: recumbent; unable to rise; euthanasia often indicated.

3.3.5. Haematological observations

Blood samples were collected in vials containing the anticoagulant sodium fluoride powder, from calves of both the groups before treatment and thereafter on the fifth and fifteenth post-treatment days and the estimation of haemoglobin concentration (Hb) (Sahli's method), volume of packed red cells (VPRC) (Wintrobe method), total leukocyte count and differential leukocyte counts (Benjamin, 1985) were carried out.

3.3.6. Serum biochemical observations

Serum samples were collected without anticoagulant from calves of both the groups before treatment and thereafter on the fifth and fifteenth post-treatment days and estimations of glucose and alkaline phosphatase level using standard chemical kit[5] were carried out.

3.3.7. Synovial fluid evaluation

The synovial fluid was collected via aseptic arthrocentesis of the affected joint, prior to treatment only and was subjected for physical, cytological, biochemical and culture and sensitivity tests (Fig. 7, 8 and 9).

3.3.7.1. Physical evaluation

3.3.7.1.1. Colour and odour: The colour and odour of the synovial fluid sample collected was recorded in calves of both the groups.

3.3.7.1.2. Clotting time: Clotting time of the collected synovial fluid samples without addition of any anticoagulant was recorded in calves of both the groups.

5. Agappe Diagnostics Pvt. Ltd., Ernakulam.

3.3.7.1.3. Mucin clot quality: For mucin precipitation, 0.8ml of distilled water and one drop of 2.5 per cent acetic acid was taken in a small test tube and was mixed thoroughly. To this 0.2ml of synovial fluid was added in such a way that the fluid was not coming in contact with glass of test tube. The fluid was mixed by swirling action of the test tube and was allowed to stand for one hour at room temperature (Fig.10) and interpreted according to Chauhan and Agarwal (2006) as follows:

Grade	Interpretation
I	A tight ropy clump in a clear solution-normal (N)
II	A soft mass in a slight turbid solution-fair (F)
III	A small friable mass in a turbid solution –poor (P)
IV	A few flakes present in a turbid solution-very poor (VP)

3.3.7.2. Cytological evaluation: The cytological examination was conducted for differential leukocyte count in which the percentage distribution of different leukocytes were determined. The sample was centrifuged at 3000 r.p.m (revolutions per minute) for 30 minutes. Smears were made from the sediment on glass micro slides (25 by 75 mm.) and air dried. These smears were then stained with Wright's stain for one to two minutes, neutralized with phosphate buffer for three minutes, rinsed with distilled water and air dried (Van Pelt and Conner, 1963).

3.3.7.3. Biochemical evaluation: Sufficient samples were transferred from aspirating syringes to capped serum vials and a 10 per cent aqueous solution of EDTA was used as the anticoagulant for prevention of clotting. Estimation of glucose and alkaline phosphatase levels in samples from both the groups were carried out using standard chemical kits.

3.3.7.4. Culture and sensitivity test: Synovial fluid samples were inoculated in brain-heart infusion agar and those samples wherein, growth was detected were again inoculated in McConkey agar plates. Characteristic tests for confirming *Escherichia coli* were conducted in two samples. Antibiotic sensitivity was also

3.3.8. Radiographic evaluation

Radiographic evaluation of the affected joint was carried out before treatment, on the fifth and fifteenth days postoperatively. Plain radiographs were taken in medio-lateral view. The radiographs were studied for change in soft tissue density, change in joint space and any alteration in the bone and articular cartilage.

3.4. THROUGH-AND-THROUGH NEEDLE LAVAGE

3.4.1. Instruments: Sterile 1.5 inch long 18 gauge needles and 50ml syringes were used for the lavage.

3.4.2. Portals for carpal joint: With the joint partially flexed a sterile inlet needle (ingress needle) was inserted into the dorsolateral joint capsule lateral to the tendon of extensor carpi radialis and medial to the combined tendon of two bellies of the common digital extensor muscle. To enter the radiocarpal compartment the needle was inserted between the distal portion of the radius and the proximal dorsal edge of the intermediate carpal bone. The midcarpal compartment was entered between the distal portion of the intermediate carpal bone and axial dorsal articular facet of the fourth carpal bone. The outlet needle was placed medial to the extensor carpi radialis tendon. The needle was inserted between the distal dorsal rim of the radius and proximal dorsal articular rim of the radial carpal bone for radio-carpal compartment. In the mid-carpal joint, the outlet needle (egress needle) was placed between the distal portion of the radial carpal bone and dorsal articular surface of the third carpal bone. Whenever the maximal distension of the joint was away from the recommended site, the ingress/inlet needle was inserted at the centre of maximum distension.

3.4.3. Anaesthesia and control: The calves selected for lavage were fasted overnight, and were sedated by administering xylazine hydrochloride[6] at the rate of 0.1 mg/kg body weight intramuscularly. The animals were secured in lateral recumbency with the limb to be examined above. The site and the adjacent areas were prepared by shaving, scrubbing with soap and water and rinsing with 70 per cent methyl alcohol, after which tincture iodine was painted over the area. One milliliter of two per cent lignocaine hydrochloride was infiltrated subcutaneously at the site using two centimeter 21 gauge needle. Two of the calves (II_1 and II_3) included in the study were highly compromised. Hence xylazine hydrochloride was not administered. Instead an additional one milliliter of two per cent lignocaine was infiltrated into the deeper layers of the fibrous joint capsule.

3.4.4. Technique: A sterile ingress needle was introduced into the joint through ingress portal as described above. The needle was advanced into the joint by taking care to avoid direct contact whenever possible with bones until some spontaneous flow of fluid occurs from the joint. A second sterile needle of same size was introduced through the egress portal in the same way as mentioned above. Dimethyl sulfoxide (DMSO) was the solution used for the joint lavage (Fig.11). A 10 per cent solution of DMSO was made by diluting with sterile normal saline solution. The lavage was performed using a 50ml syringe so as to create turbulence and dislodge fibrin particles adhered to the synovial membrane. Lavage was continued until the egress fluid was some what clear. Whenever the joint was highly open, the egress fluid was allowed to exit through the wound opening (Fig. 12). The depth of the needle was adjusted to ensure good flow of fluid. The outflow tract was frequently occluded so as to distend the joint. At the end of lavage, the fluid was manually expressed from the joint. Once the residual fluid was expressed, the limb was bandaged using elastic bandage after covering the joint with a sterile cotton pad. The same therapy was repeated on the fifth day in animals that had persistent effusion.

6. Xylaxin- (Xylazine hydrochloride 23.32 mg/ml, equivalent to Xylazine 20mg/ml) Indian Immunologicals LTD., Hyderabad.

3.5. HISTOPATHOLOGY

Histopathological evaluation of the affected knee joint was performed in two calves of Group II which succumbed during the observation period; using routine Haematoxylin and Eosin (H&E) staining and both the macroscopical and microscopical lesions were recorded.

3.6. OTHER OBSERVATIONS, IF ANY

Observations regarding outcome of treatment modality along with the complications occurred were recorded in calves of both the groups.

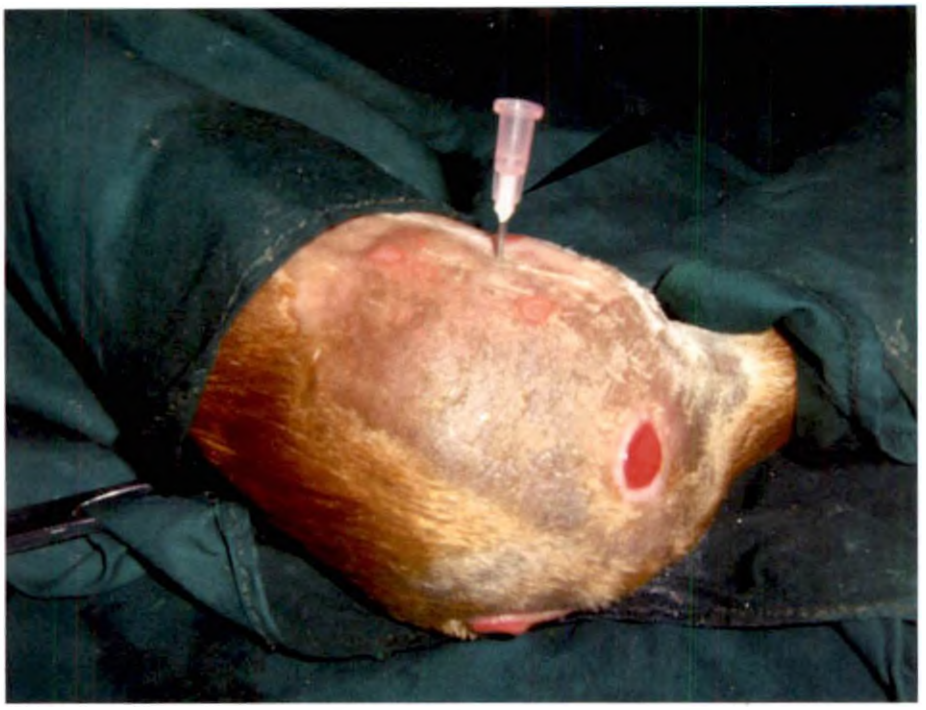


Fig.1. Ingress needle for through-and -through lavage in position

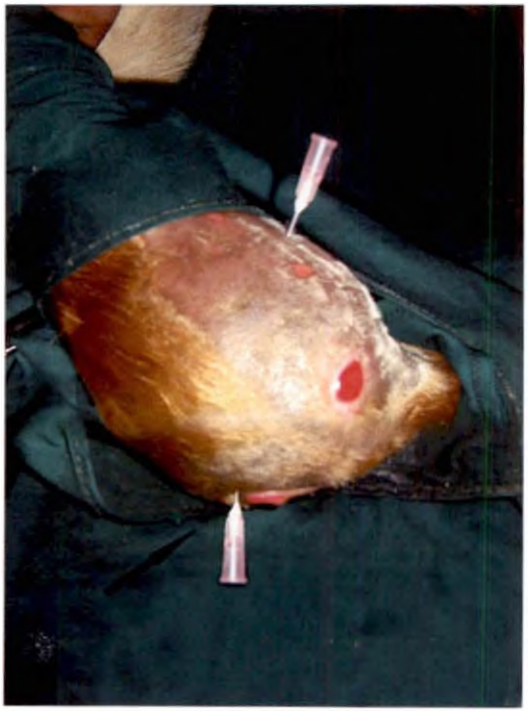


Fig.2.Egress needle for through-and -through lavage in position

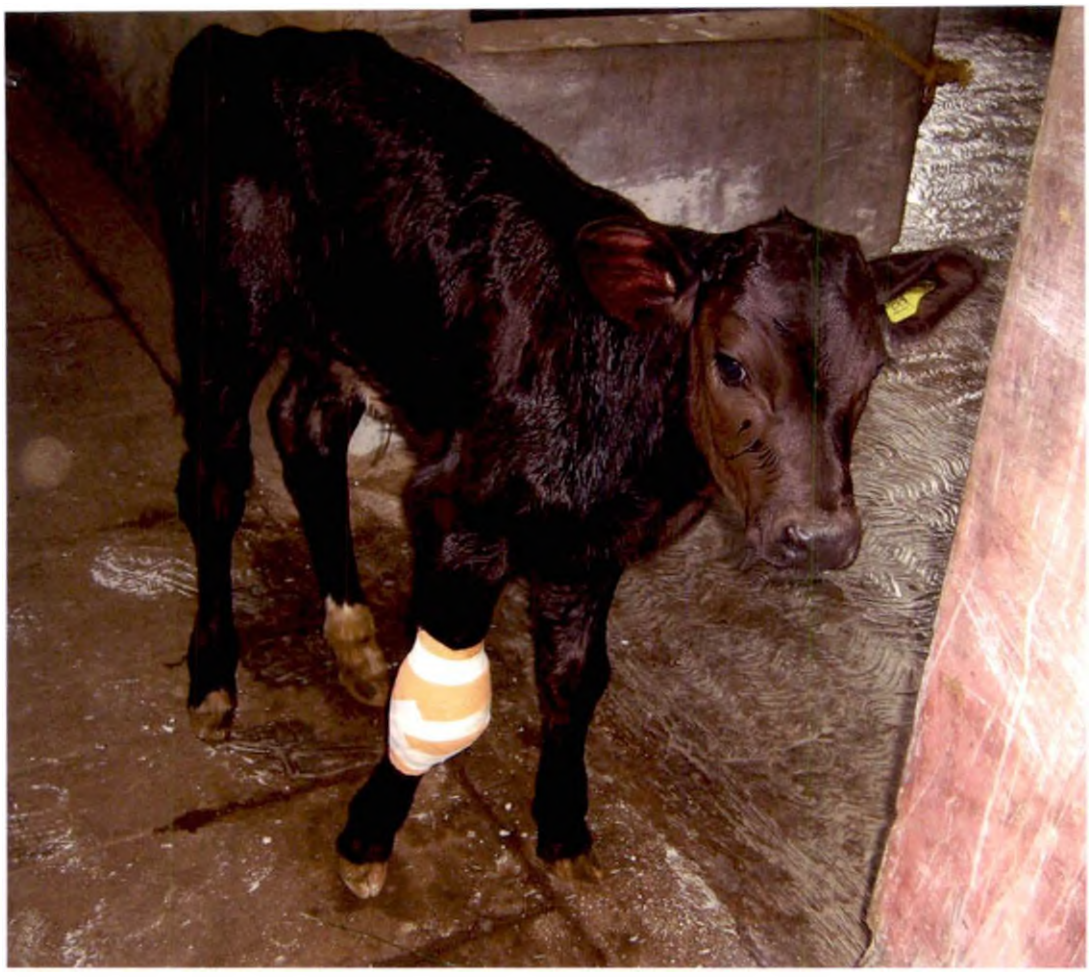


Fig.3. Knee joint in a calf (II_2)after lavage, with elastic crepe bandage.

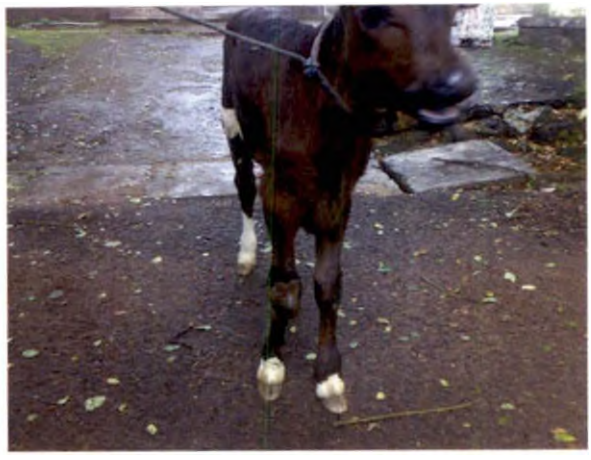


Fig.4.Swollen knee joint in a calf (I_2)

Fig.5. Open knee joint in a calf (II_2

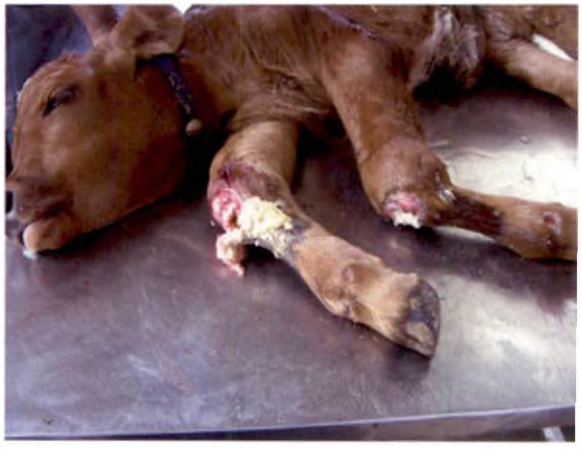


Fig.6.Open knee joints in a calf (II_1) with necrotic tissues

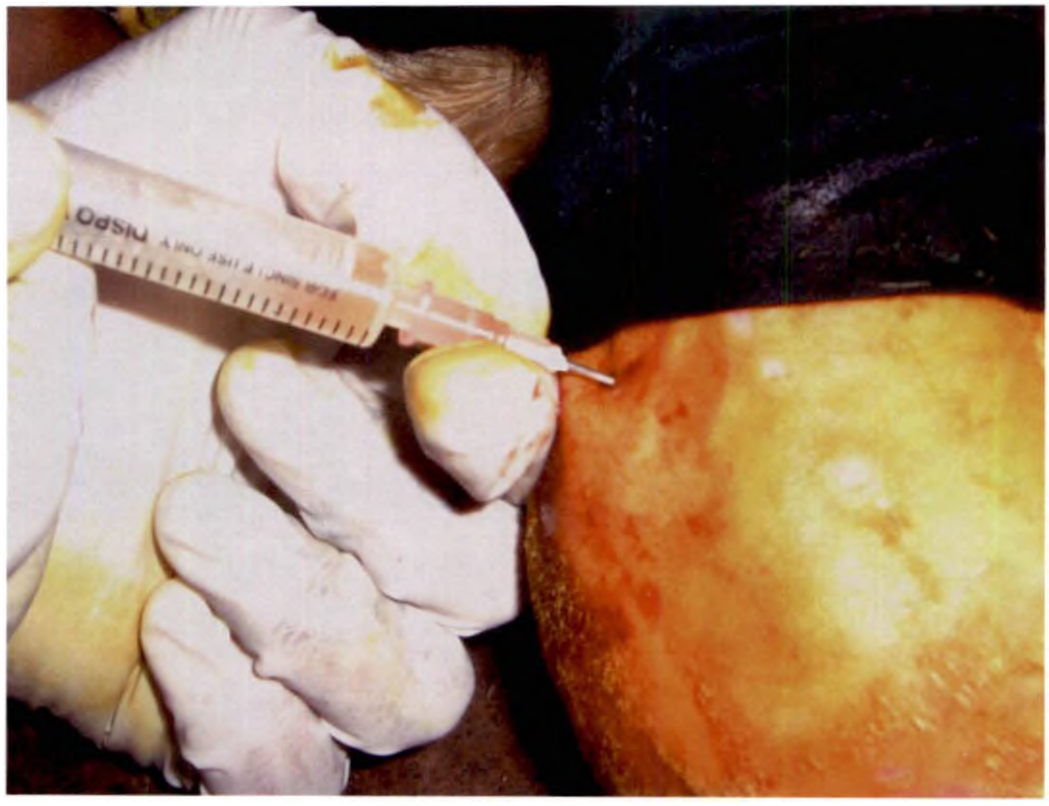
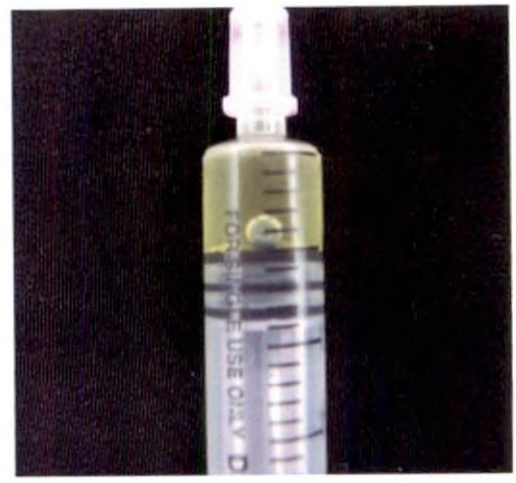


Fig.7. Arthrocentesis for collection of synovia

Fig.8. Non - purulent synovia in a syringe



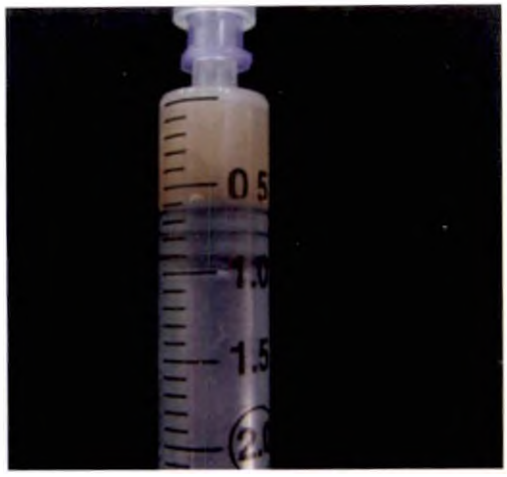
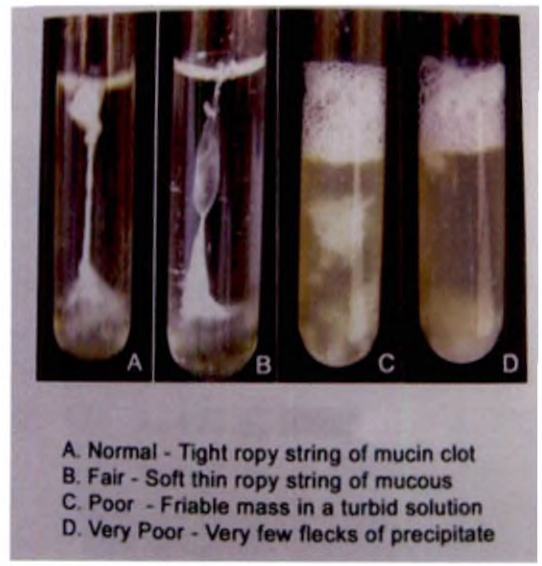


Fig.9. Purulent synovia in a syringe

Fig.10. Synovial fluid - Mucin clot test



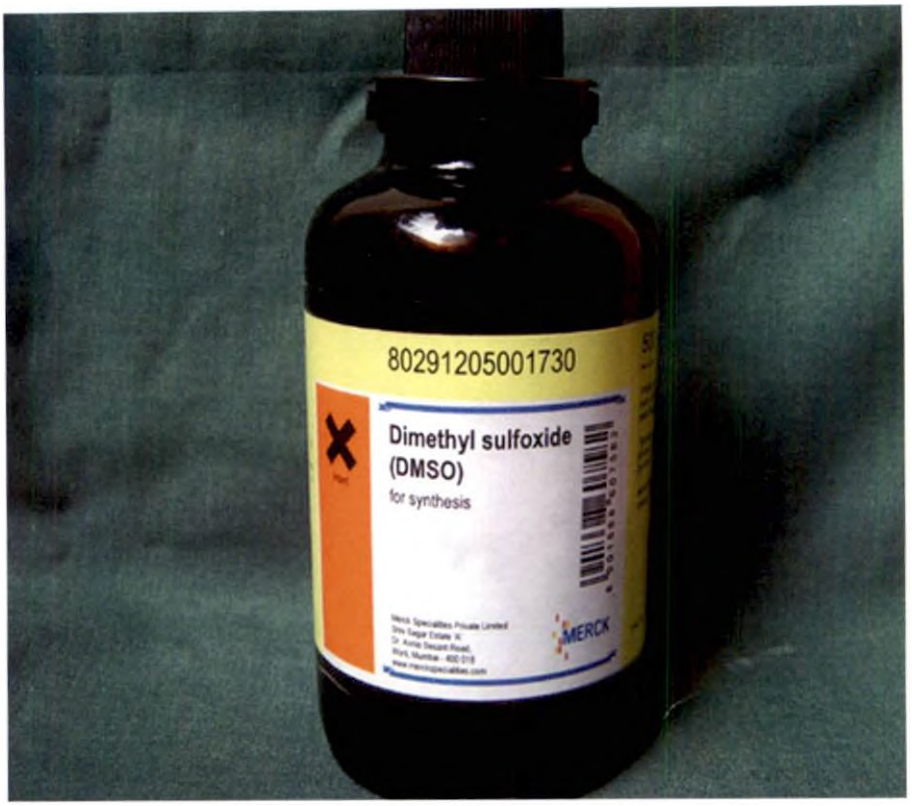


Fig.11. Dimethyl sulfoxide (DMSO)

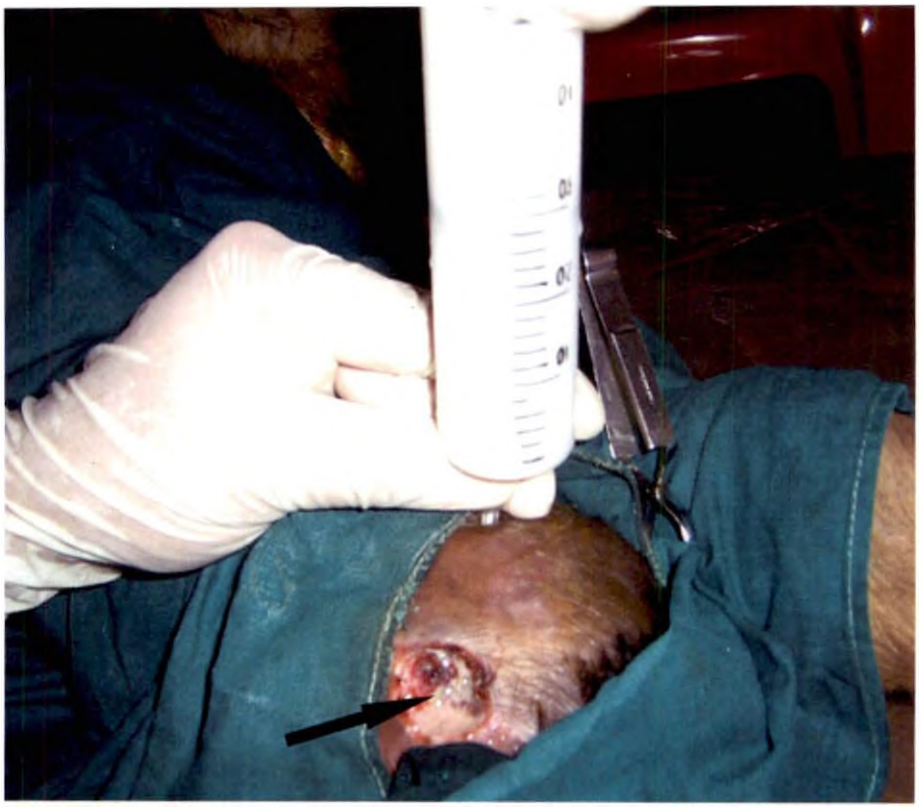


Fig.12. Lavage fluid exiting through the open wound-calf (II_3)

4. RESULTS

The study was carried out in twelve calves affected with arthritis aged below six months belonging to different breeds of either sex, presented to the College Veterinary Hospital at Mannuthy and University Veterinary Hospital at Kokkalai. Details about signalment and anamnesis were collected. All the calves were subjected to detailed clinical, physiological, haematological and serum biochemical evaluation. Radiographic and synovial fluid evaluations of the affected joint(s) were also carried out. Based on the nature of synovia, whether it was non-purulent or purulent, the calves were divided into Group I and II respectively consisting of six calves each.

4.1. GROUP I

4.1.1. Signalment (Table. 1)

***4.1.1.1. Age*:** The age of calves ranged from five days to two months.

***4.1.1.2. Breed*:** The breeds affected were Holstein-Friesian cross (I_3, I_4 and I_5) and Jersey cross bred (I_1, I_2 and I_6) with three animals each.

***4.1.1.3. Sex*:** The male (I_3, I_4 and I_6) and female (I_1, I_2 and I_5) calves were three each in this group.

***4.1.1.4. Joint(s) affected*:** The only joint affected was the knee joint and was unilateral. In four calves (I_2, I_4, I_5 and I_6), the right and in two ($I_1$and I_3) the left joints were involved.

4.1.2. Anamnesis

Four calves (I_1, I_2, I_4 and I_6) in this group had the history of umbilical infection. The umbilical cords of these calves were torn naturally at the time of birth and the stumps were left untreated. The duration of illness ranged from three days to one week. Two calves (I_2 and I_4) were treated for umbilical

infection, previously by surgical drainage, local dressing and antibiotic therapy with Streptomycin- penicillin combination. Two calves (I_1 and I_6) were not sufficiently fed with colostrum within half an hour of calving. All calves were with slight degree of lameness and were not dewormed. In all the calves the knee joint was affected and the duration of illness ranged from three days to one week.

4.1.3. Clinical observations

Subjective monitoring of the affected joint was performed. The joints were swollen, warm to touch and evidenced pain upon palpation in all calves (Fig. 13). The skin of these joints was remaining intact in all calves. Deviation from normal posture and gait was evidenced in these animals at the time of presentation to the hospital. Pointing of toe was noticed in the affected limb in three calves (I_1, I_3 and I_5). In all the calves, the affected limb could be flexed and evidenced pain while flexing.

The joint swelling was reduced by the fifth day of observation in two calves (I_4 and I_5). In the other three calves, the swelling and warmth of joint was almost absent by the fifteenth day. But in one calf (I_1) the swelling and warmth of joint persisted till two months; after which it was reduced. Pointing of toe was absent by the fifteenth day of observation in I_2 (Fig. 14), I_3 and I_5. Pain on palpation of affected joint was absent by the fifth day in I_5 and in the other calves by the fifteenth day.

All calves exhibited mild degree of lameness before treatment (Table. 2) and during the observation period of fifteen days, except in two calves (I_2 and I_3) which exhibited moderate degree of lameness. I_2 improved its condition by the fifteenth day becoming mildly lame. No alteration in degree of lameness was noticed in I_3.

4.1.4. Physiological observations (Table.3)

The rectal temperature (°C) was 39.27±0.19 before treatment and it was 38.72±0.13 and 38.33±0.17 on the fifth and fifteenth day respectively.

The pulse rate (per min.) was 73.17±1.22 before treatment and it was 73±1.24 and 73.17±0.83 on the fifth and fifteenth day respectively.

The respiration rate (per min.) was 18.33±1.98 before treatment and it was 18.5±2.02 and 19.17±1.76 on the fifth and fifteenth day respectively.

4.1.5. Haematological observations (Table. 4)

***4.1.5.1. Haemoglobin concentration*:** The mean haemoglobin concentration (g/dl) was 10.23±0.23 before treatment and it was 11.07±0.48 and 10.86±0.53 on the fifth and fifteenth day respectively.

***4.1.5.2. Volume of packed red cells (VPRC)*:** The mean packed cell volume (per cent) was 27.77±1.56 before treatment and it was 26.8±1.90 and 26.83±2.29 on the fifth and fifteenth day respectively.

***4.1.5.3. Total leukocyte count (TLC)*:** The mean total leukocyte count (10^3/ cu.mm) was 16.15±0.96 before treatment and it was 10.19±0.81 and 7.07±0.54 on the fifth and fifteenth day respectively.

4.1.5.4. Differential leukocyte count (DLC)

Neutrophil count (per cent): It was 70.5±5.69 before treatment and 49.33±5.21 and 41.67±3.53 on the fifth and fifteenth day respectively.

Lymphocyte count (per cent): It was 27.5±5.23 before treatment and 48.83±4.92 and 57.17±3.22 on the fifth and fifteenth day respectively.

Eosinophil count (per cent): It was 2.00±0.45 before treatment and 2.20±0.49 and 2.20±0.49 on the fifth and fifteenth day respectively.

4.1.6. Serum biochemical observations (Table. 5)

***4.1.6.1. Glucose*:** The mean serum glucose (mg/dl) level before treatment was 115.67±7.50 and it was 93.50±4.02 and 95.83±3.91 on the fifth and fifteenth day respectively.

***4.1.6.2. Alkaline phosphatase*:** The mean alkaline phosphatase (U/L) level before treatment was 448±8.40 and it was 395.20±6.34 and 345.67±10.70 on the fifth and fifteenth day respectively.

4.1.7. Synovial fluid evaluation

***4.1.7.1. Physical evaluation*:** The colour was slightly yellow in all calves. All collected samples were odourless. The mucin clot quality was grade I or normal (N) except in I_2 and I_3 which was grade II or fair (F). There was clotting of synovial fluid sample within ten minutes in I_2 and the rest of the samples did not clot under standard conditions. (Table. 6)

4.1.7.2. Cytological evaluation

4.1.7.2.1. Differential leukocyte count: The mean neutrophil count (per cent) was 68.50±8.31 and the mean lymphocyte count was 31.50±8.31.

4.1.7.3. Biochemical observations

4.1.7.3.1. Glucose: The mean synovial fluid glucose level (mg/dl) was 83.33±7.53.

4.1.7.3.2. Alkaline phosphatase: The mean alkaline phosphatase level (U/L) was 800.50±174.02.

***4.1.7.4. Culture and sensitivity test*:** Of the six samples taken, no isolates were obtained after 48 hours of incubation on Bovine Heart Infusion Agar (BHIA). One isolate of *Escherichia coli* was obtained from the umbilical pus in one calf (I_1) and the organism was found to be sensitive to gentamicin.

4.1.8. Radiographic evaluation

The observations of the lateral plain radiographs of the affected knee joints were recorded before treatment and on the fifth and fifteenth days after treatment. The details are given below:

Calf I_1: Moderate soft tissue swelling was the major lesion observed and no gross changes were observed in the bone or cartilage in the radiograph before treatment. Fifth and fifteenth day radiographs revealed no changes/alterations in the joint (Fig. 15 and 16).

Calf I_2: The major lesion was the moderate soft tissue swelling in the pre-treatment radiograph. The fifth day radiograph does not reveal any alteration. Considerable reduction of soft tissue swelling in the fifteenth day radiograph was observed.

Calf I_3: Moderate soft tissue swelling was the major lesion observed. Alterations from the initial radiograph were not observed in the fifth day radiograph and much reduction of swelling observed in the fifteenth day radiograph.

Calf I_4: Characteristic radiographic abnormalities could not be detected in the radiographs taken before treatment and on the fifth and fifteenth day.

Calf I_5: Mild soft tissue swelling could be detected in the pre-treatment radiograph and absent in the fifteenth day.

Calf I_6: Mild soft tissue swelling was the major lesion detected and was absent in the fifth and fifteenth day post-treatment radiographs (Fig. 17, 18 and 19).

4.1.9. Other observation, if any

Out of the six calves treated three (I_4, I_5 and I_6) fully recovered with single intra-articular therapy, another three (I_1, I_2 and I_3) with repeated intra-articular therapy on the fifth day.

4.2. GROUP II

4.2.1. Signalment (Table. 1)

***4.2.1.1. Age*:** The age of calves ranged from five days to one month.

***4.2.1.2. Breed*:** The breeds affected were one Holstein-Friesian cross (II_2) and five Jersey cross bred (II_1, II_3, II_4, II_5 and II_6).

***4.2.1.3. Sex*:** The male (II_1) and female (II_2, II_3, II_4, II_5 and II_6) calves were one and five respectively in this group.

***4.2.1.4. Joint(s) affected*:** The joint included in the study was the knee joint. In three calves (II_2, II_3 and II_4), the right and in one (II_6) the left joints were involved. In two calves (II_1 andII_5) both right and left knee joints were involved.

4.2.2. Anamnesis

The calf (II_1) was recumbent and was debilitated at the time of presentation to the hospital. No umbilical infection was noticed in any of the calves. II_1 was previously treated with sulpha bolus and colostrum intake was not sufficient in II_1. The rest of the animals were healthy at the time of presentation except II_3 which showed slight respiratory distress. In all the calves the knee joint was affected and the duration of illness ranged from two days to one week. None of the calves were dewormed at the time of presentation to the hospital.

4.2.3. Clinical observations

The affected knee joints were swollen, warm to touch and evidenced pain on palpation in all calves at the time of presentation to the hospital. Exudations were present in all the calves with open joints. Deviation from normal posture and gait was observed in all calves. One calf (II_1) was presented with the animal recumbent and unable to stand, with necrotic tissues protruding out from the open joints (Fig. 20) in both knee joints. Flexing of affected joint was difficult

in three calves (II_1, II_3 and II_6). Pointing of toe and ankylosis of the affected joint was present in two calves (II_3 and II_6).

The joint swelling and pus was reduced by the fifth day of observation in two calves (II_2 and II_4) (Fig. 21) and further improved by the fifteenth day (Fig.22). In other calves the swelling persisted throughout the period of observation. Open joint healed by the fifteenth day in II_4 and by one month in II_2 (Fig. 23). Pointing of toe persisted in the affected calves throughout the period of observation. Pain on palpation of the affected joint was absent by the fifteenth day in II_2 and II_4 and improvement was noticed in II_1 (Fig. 24). Ankylosis in II_3 and II_6 persisted throughout the observation period.

II_1 was always recumbent and unable to rise throughout the period of observation with the lameness score of 4. II_2 and II_4 were mildly lame throughout the period of observation with the score of 1. II_3 was severely lame throughout the observation period with the score of 3. II_5 was moderately lame at the time of presentation with a score of 2 and became catastrophe by the fifth day with the score of 4 throughout the observation period. II_6 was severely lame throughout the observation period with a score of 3 (Table. 2).

4.2.4. Physiological observations (Table. 3)

The rectal temperature (°C) was 39.26±0.20 before treatment and it was 38.94±0.24 and 38.42±0.18 on the fifth and fifteenth day respectively.

The pulse rate (per min.) was 78.66±1.84 before treatment and it was 80.33±2.28 and 80.40±1.83 on the fifth and fifteenth day respectively.

The respiration rate (per min.) was 25.50±0.76 before treatment and it was 25.12±0.31 and 25±0.31 on the fifth and fifteenth day respectively.

4.2.5. Haematological observations (Table. 4)

***4.2.5.1. Haemoglobin concentration*:** The mean haemoglobin concentration (g/dl) was 10.65±0.57 before treatment and it was 10.33±0.60 and 9.7±0.24 on the fifth and fifteenth day respectively.

***4.2.5.2. Volume of packed red cells (VPRC)*:** The mean packed cell volume (per cent) was 31.5±2.52 before treatment and it was 31±2.62 and 28.08±2.07 on the fifth and fifteenth day respectively.

***4.2.5.3. Total leukocyte count (TLC)*:** The mean total leukocyte count (10^3/ cu.mm) was 16.03±1.14 before treatment and it was 13.47±1.35 and 9.33±1.37 on the fifth and fifteenth day respectively.

4.2.5.4. Differential leukocyte count (DLC)

Neutrophil count (per cent): It was 64.33±7.04 before treatment and 55.83±6.61 and 39.8±9.95 on the fifth and fifteenth days respectively.

Lymphocyte count (per cent): It was 33.50±6.81 before treatment 42.83±6.53 and 57.20±9.09 on the fifth and fifteenth days respectively.

Eosinophil count (per cent): It was 1.33±0.42 before treatment and 0.50±0.22 and 0.60±0.40 on the fifth and fifteenth days respectively.

Monocyte count (per cent): It was 0.83±0.40 before treatment and 0.83±0.40 and 2.40±1.17 on the fifth and fifteenth days respectively.

4.2.6. Serum biochemical observations (Table. 5)

***4.2.6.1. Glucose*:** The mean serum glucose (mg/dl) level before treatment was 88.83±12.76 and it was 92.83±11.29 and 99.40±12.24 on the fifth and fifteenth day respectively.

***4.2.6.2. Alkaline phosphatase*:** The mean alkaline phosphatase (U/L) level before treatment was 305±6.71 and it was 298.17±7.22 and 280.40±11.03 on the fifth and fifteenth day respectively.

4.2.7. Synovial fluid evaluation

***4.2.7.1. Physical evaluation*:** The colour was turbid yellow along with some flocculent materials in all calves. The mucin clot quality was very poor in two calves (II_1 and II_5), was poor in two calves (II_2 and II_3) and was fair in two calves (II_4 and II_6). The collected samples were odourless in four calves (II_2, II_3, II_4, and II_6) and were having a pungent odour in two calves (II_1 and II_5). There was clotting of synovial fluid sample within five minutes in II_1 and II_5, within ten minutes in II_2, within seven minutes in II_3, within twenty minutes in II_4 and within one hour in II_6. (Table. 6)

4.2.7.2.. Cytological evaluation

***4.2.7.2.1. Differential leukocyte count*:** The mean neutrophil count (per cent) was 85.67±1.09 and the mean lymphocyte count was 14.33±1.09. (Fig. 25)

4.2.7.3. Biochemical observations

***4.2.7.3.1. Glucose*:** The mean synovial fluid glucose level (mg/dl) was 26.33±11.07.

***4.2.7.3.2. Alkaline phosphatase*:** The mean alkaline phosphatase level (U/L) was 970±93.91.

***4.2.7.4. Culture and sensitivity test*:** Of the six samples taken, two isolates of *Escherichia coli* and characteristic pink colonies on Mc Conkey agar plates were obtained. Sulphadiazine was sensitive in one isolate and gentamicin in the other one. (Fig. 26)

4.2.8. Radiographic evaluation

The observations of the lateral plain radiographs of the affected knee joints were recorded before treatment and on the fifth and fifteenth days after treatment. The details are given below:

Calf II_1: Severe peri-articular soft tissue swelling with increased radio-carpal joint space was observed in the pre-treatment radiographs of left and right knee joints. Considerable changes from the initial radiographs were not observed in the fifth and fifteenth post-treatment radiographs. (Fig. 27 and 28)

Calf II_2: Moderate peri-articular soft tissue swelling was observed on the right knee joint in the pre-treatment radiograph and considerable alterations from the initial radiograph were not observed in the fifth and fifteenth post-treatment radiographs.

Calf II_3: Severe peri-articular soft tissue swelling in the right knee joint with increased radio-carpal and mid-carpal joint space. Gas shadow was observed in the radio-carpal and mid-carpal joints. Blurring of normal bone outline and subchondral osteolysis was noticed in the proximal metacarpal. (Fig.29)

Calf II_4: Mild soft tissue swelling was the major lesion observed in the pre-treatment radiograph and it was almost resolved in the fifteenth day post-treatment radiograph.

Calf II_5: Severe peri-articular soft tissue swelling in the right and left knee joints with gas shadow in the right knee joint and widening of radio-carpal and carpo-metacarpal joint spaces were observed in the pre-treatment radiograph. The only change observed in the fifteenth day radiograph was that there was considerable reduction in the peri-articular soft tissue swelling.

Calf II_6: Moderate peri-articular soft tissue swelling in the left knee joint with severe periosteal reaction and widening of carpo-metacarpal joint space. Blurring

of normal bone outline was also noticed in the pre-treatment radiograph. Much change was not observed in the fifteenth day radiograph. (Fig. 30 and 31).

4.2.9. Histo-pathological findings

The affected joints of the two calves which succumbed during the observation period were harvested and subjected to macroscopical and microscopical examination. Macroscopically the joints were swollen and on opening, purulent discharge was noticed along with cartilage degeneration (Fig.32). Microscopic examination revealed, the sections showing thick hyalinised fibrocollagenous tissue with dense infiltrate of inflammatory cells, areas of hemorrhage, and many congested blood vessels (Fig. 33 and 34). Some areas appeared like abscess wall of which was formed by inflammatory granulation tissue. One section showed bony tissue with degenerating bony trabeculae. Periosteum and adjacent tissues showed extensive necrosis and abscess formation.

4.2.10. Other observation, if any

In neither of the calves, single lavage using 10 per cent DMSO proved to be effective except in I_4 in which only single lavage was performed with good results. In other calves the lavage was repeated on the fifth day. II_1 and II_3 succumbed into death; II_1 one month after treatment and II_3 within seven days after the initiation of treatment. Pus in the affected joints considerably reduced by the fifteenth day after the lavage in four calves (II_1, II_2, II_4 and II_6). Ankylosis of the affected joint in II_3 and II_6 could not be resolved.

Table.1. Summary of signalment of Group I and II calves.

Group	Calf No.	Breed	Sex	Age	Knee joint
I	1	Jersey cross	Female	15 days	Left
	2	Jersey cross	Female	1 month	Right
	3	Holstein Friesian cross	Male	1 month	Left
	4	Holstein Friesian cross	Male	2 months	Right
	5	Holstein Friesian cross	Female	5 days	Right
	6	Jersey cross	Male	17 days	Right
II	1	Jersey cross	Male	7 days	Right and left
	2	Holstein Friesian cross	Female	5 days	Right
	3	Jersey cross	Female	15 days	Right
	4	Jersey cross	Female	14 days	Right
	5	Jersey cross	Female	15 days	Right and left
	6	Jersey cross	Female	1 month	Left

Table. 2. Lameness scores of Group I and II calves.

Group	Calf No.	Before treatment	5th day	15th day
I	1	1	1	1
	2	2	2	1
	3	2	2	2
	4	1	1	1
	5	1	1	1
	6	1	1	0
II	1	4	4	4
	2	1	1	1
	3	3	3	-
	4	1	1	1
	5	2	4	4
	6	3	3	3

0- Normal gait; 1- Mild lameness; 2- Moderate lameness; 3- Severe lameness, 4- Catastrophe.

Table.3. Observations on Physiological Parameters in Group I and II (Mean ±SE)

Group	Parameters	Before treatment	5th day	15th day
I	Rectal temperature (°C)	39 ± 0.19	38 ± 0.13	38.33 ± 0.17
	Pulse Rate (per min.)	73.17 ± 1.22	73 ± 1.24	73.17 ± 0.83
	Respiration Rate (per min.)	18.33 ± 1.98	18.5 ± 2.02	19.17 ± 1.76
II	Rectal temperature (°C)	39.26 ± 0.20	38.94 ± 0.24	38.42 ± 0.18
	Pulse Rate (per min.)	78.66 ± 1.84	80.33 ± 2.28	80.40 ± 1.83
	Respiration Rate (per min.)	25.5 ± 0.76	25.12 ± 0.31	25 ± 0.31

Table. 4. Observation on Haematological Parameters in Group I and II (Mean± SE)

Group	Parameters	Before treatment	5th day	15th day
I	Haemoglobin concentration (g/dl)	10.23±0.23	11.07±0.48	10.86±0.53
	Volume of Packed Red Cells (per cent)	27.77±1.56	26.8±1.9	26.83±2.29
	Total Leukocyte Count (10^3/mm^3)	16.15±0.96	10.19±0.81	7.07±0.54
	Neutrophil (per cent)	70.5±5.69	49.33±5.21	41.67±3.53
	Lymphocyte (per cent)	27.5±5.23	48.83±4.92	57.17±3.22
	Eosinophil (per cent)	2±0.45	2.2±0.49	2.2±0.49
II	Haemoglobin concentration (g/dl)	10.65±0.57	10.33±0.60	9.7±0.24
	Volume of Packed Red Cells (per cent)	31.5±2.52	31±2.62	28.08±2.07
	Total Leukocyte Count (10^3/mm^3)	16.03±1.14	13.47±1.35	9.33±1.37
	Neutrophil (per cent)	64.33±7.04	55.83±6.61	39.8±9.95
	Lymphocyte (per cent)	33.5±6.81	42.83±6.53	57.2±9.09
	Eosinophil (per cent)	1.33±0.42	0.5±0.22	0.6±0.40
	Monocyte (per cent)	0.83±0.40	0.83±0.40	2.4±1.17

Table. 5. Serum Biochemical Observation of Group I and II (Mean ± SE)n=6

Group	Parameters	Before treatment	5th day	15th day
I	Glucose (mg/dl)	115.67±7.50	93.5±4.02	95.83±3.91
	Alkaline phosphatase (U/L)	448±8.40	395.2±6.34	345.67±10.70
II	Glucose (mg/dl)	88.83±12.76	92.83±11.29	99.4±12.24
	Alkaline phosphatase (U/L)	305±6.71	298.17±7.22	280.40±11.03

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Table.6. Physical evaluation of synovial fluid of Group I and II calves.

Group	Animal No.	Colour	Odour	Mucin clot quality	Clotting time
I	1	Slight yellow	Odour less	N	Ten minutes
	2	Slight yellow	Odour less	F	--
	3	Slight yellow	Odour less	N	--
	4	Slight yellow	Odour less	N	--
	5	Slight yellow	Odour less	N	--
	6	Slight yellow	Odour less	N	--
II	1	Turbid yellow with flocculents	Pungent odour	VP	Five minutes
	2	Turbid yellow with flocculents	Odour less	P	Ten minutes
	3	Turbid yellow with flocculents	Odour less	P	Seven minutes
	4	Turbid yellow with flocculents	Odour less	F	Twenty minutes
	5	Turbid yellow with flocculents	Pungent odour	VP	Five minutes
	6	Turbid yellow with flocculents	Odour less	F	One hour

N- Normal; F- Fair; P- Poor; VP- Very poor.

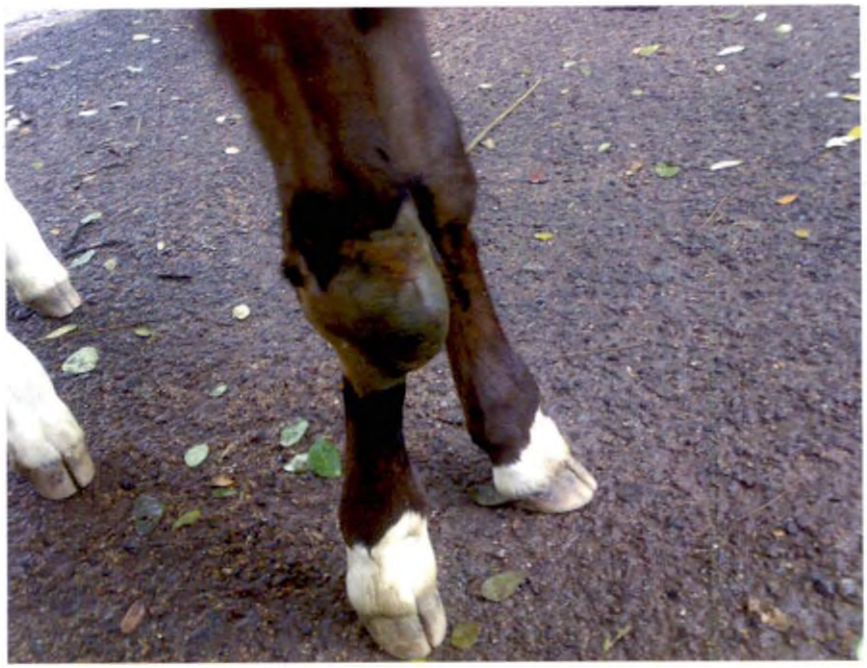

Fig.13. knee joint in a calf (I_2)- 5th day of treatment

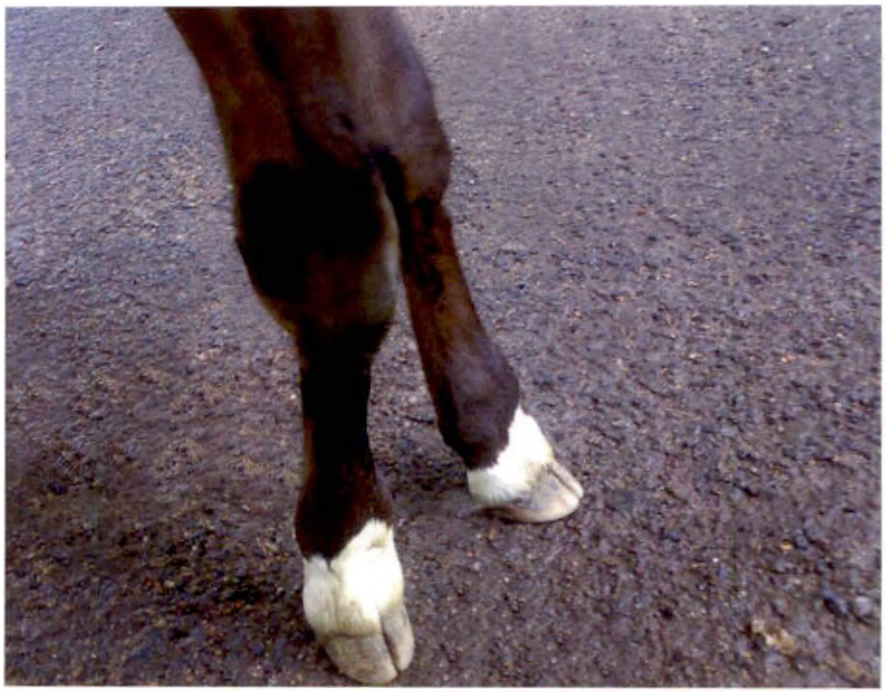

Fig.14. knee joint in a calf (I_2)- 15th day of treatment

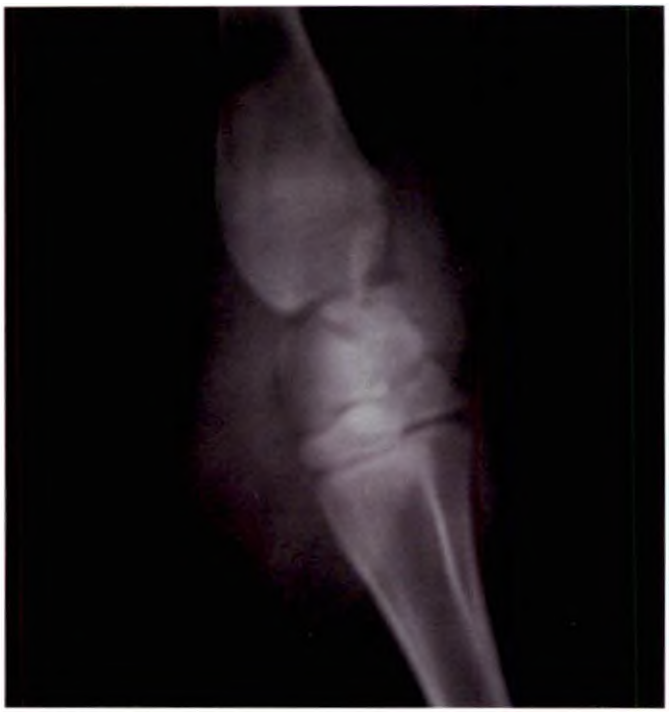

Fig.15. Skiagram of knee joint in a calf (I_1)-before treatment.

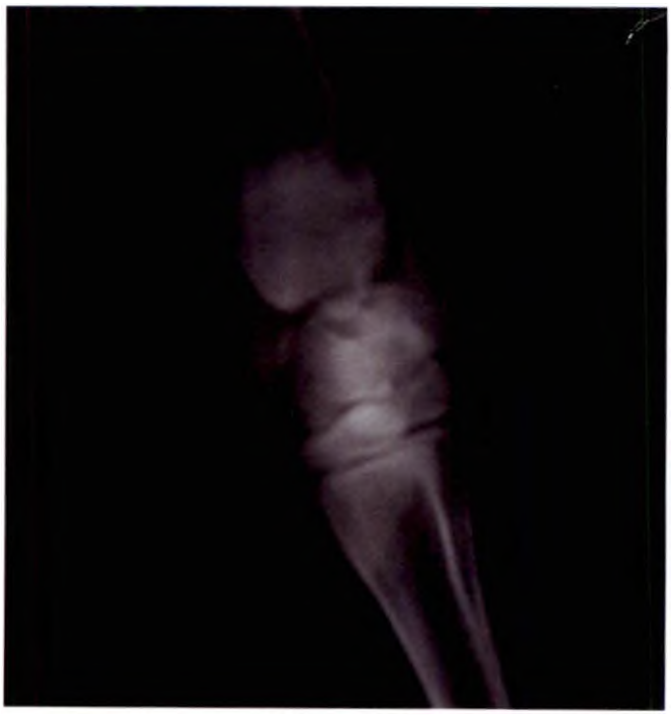

Fig.16. Skiagram of the joint in a calf(I_1) -15th day of treatment

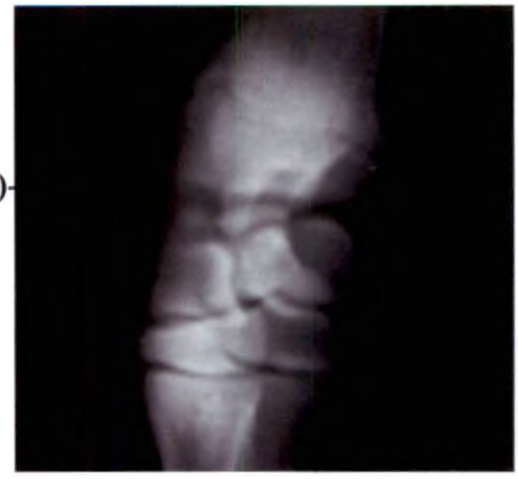

Fig.17. Skiagram of the knee joint of a calf (I_6)- Before treatment

Fig.18. Skiagram of the knee joint of a calf (I_6) -fifth day of treatment.

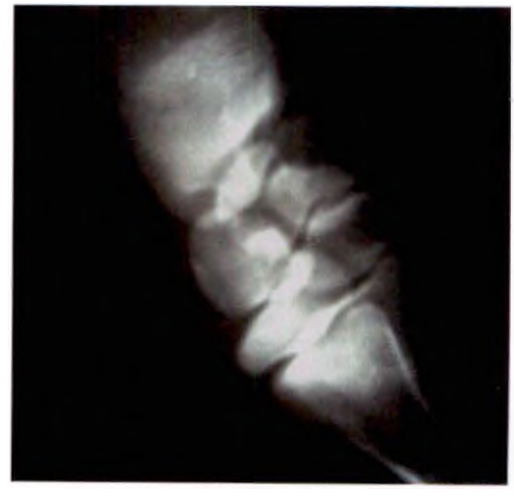

Fig.19. Skiagram of the knee joint of a calf (I_6) - 15th day of treatment.

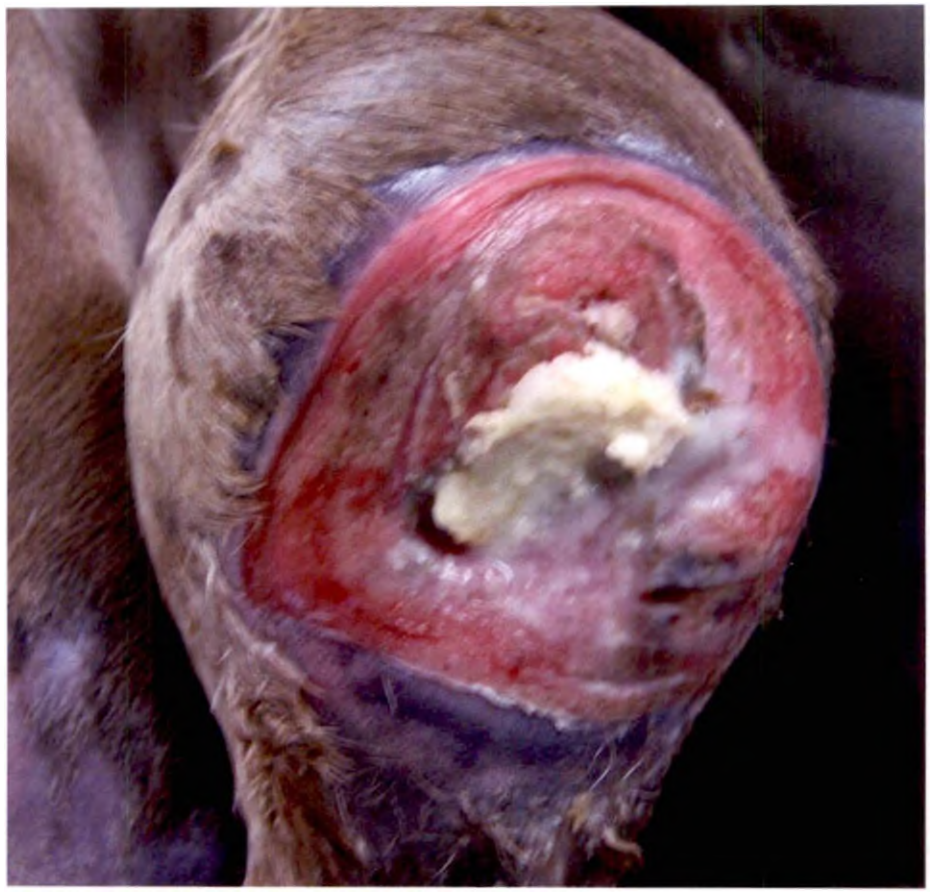

Fig.20. Knee joint in a calf (II_1)-before treatment

Fig.21. knee joint in a calf (II_2)- 5th day of treatment

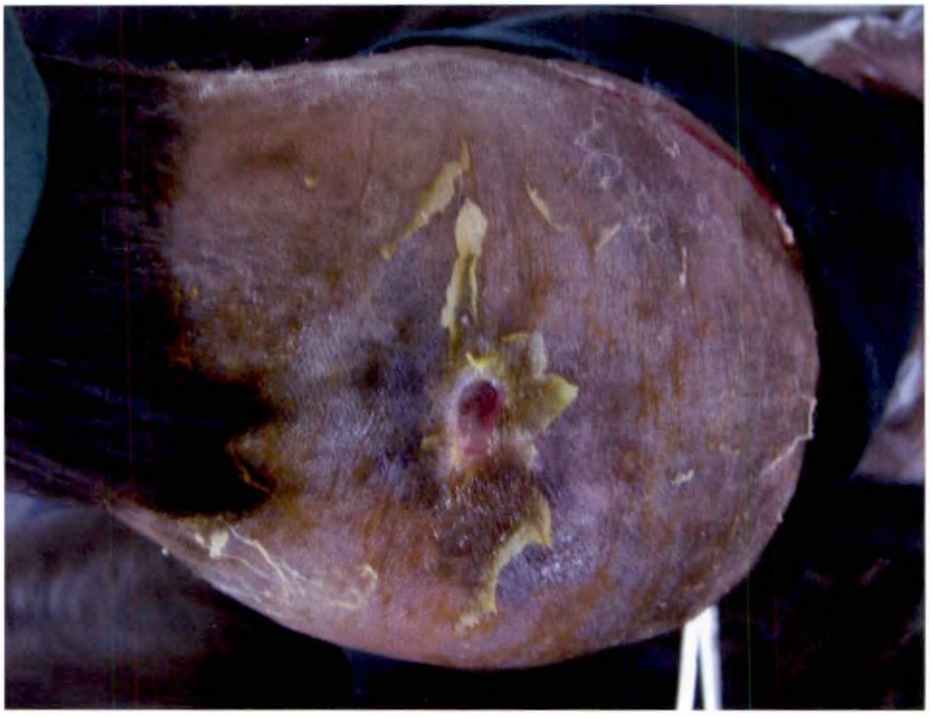

Fig.22. Knee joint in a calf (II_2)-15th day of treatment

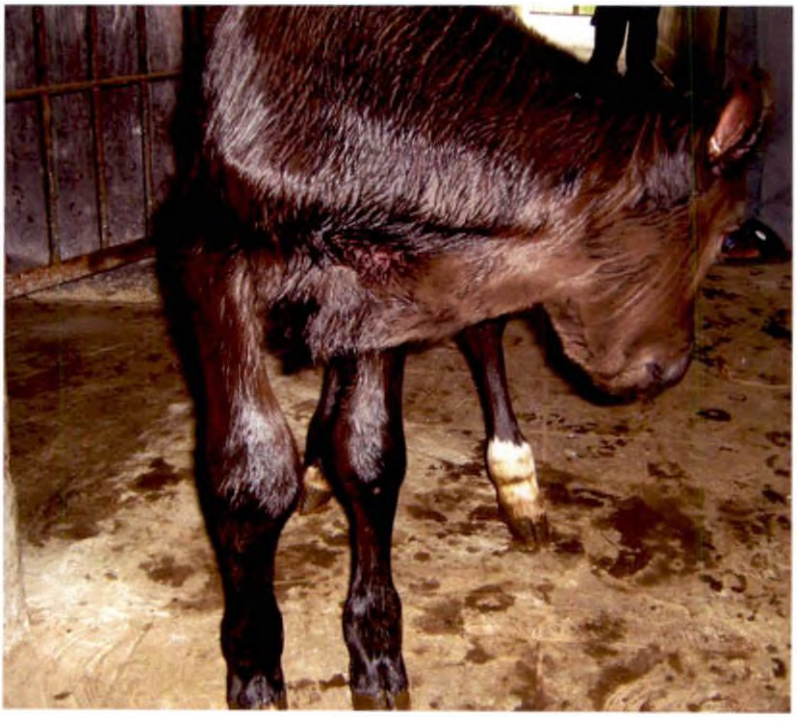

Fig.23. Knee joint in a calf (II_2)- after one month of treatment

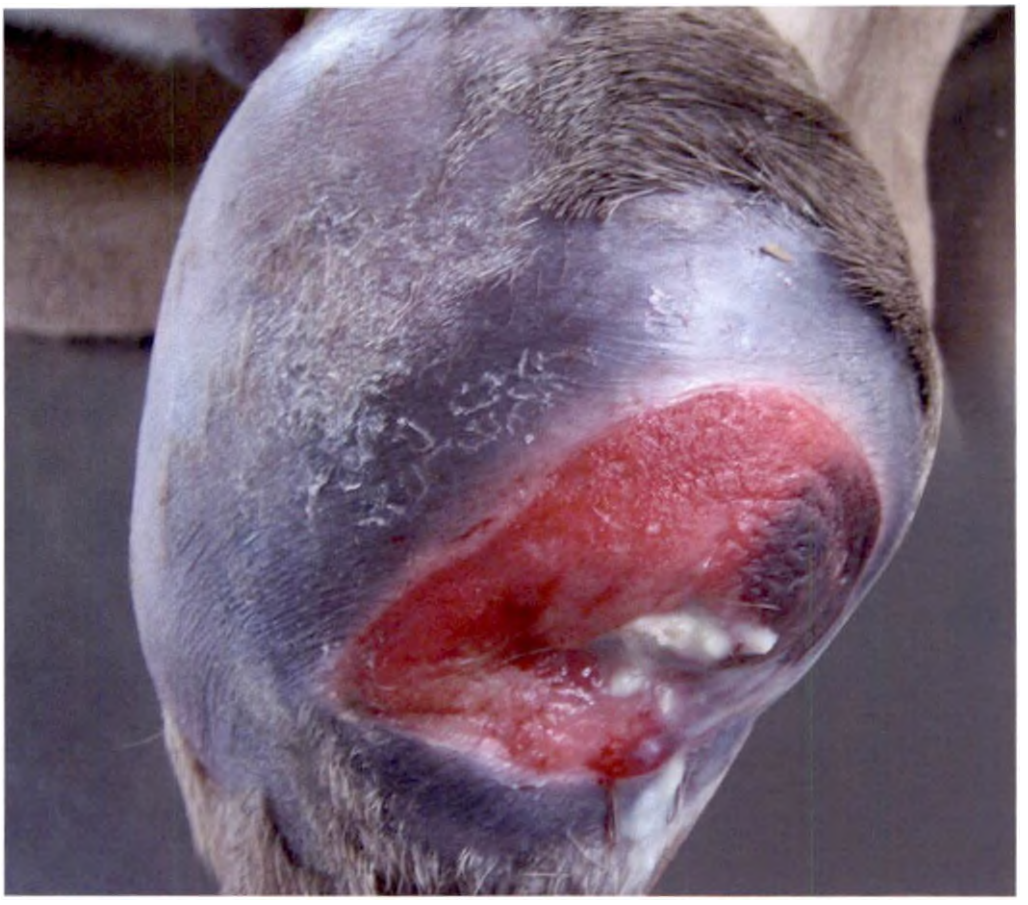

Fig.24. Knee joint in a calf (II_1)-15th day of treatment

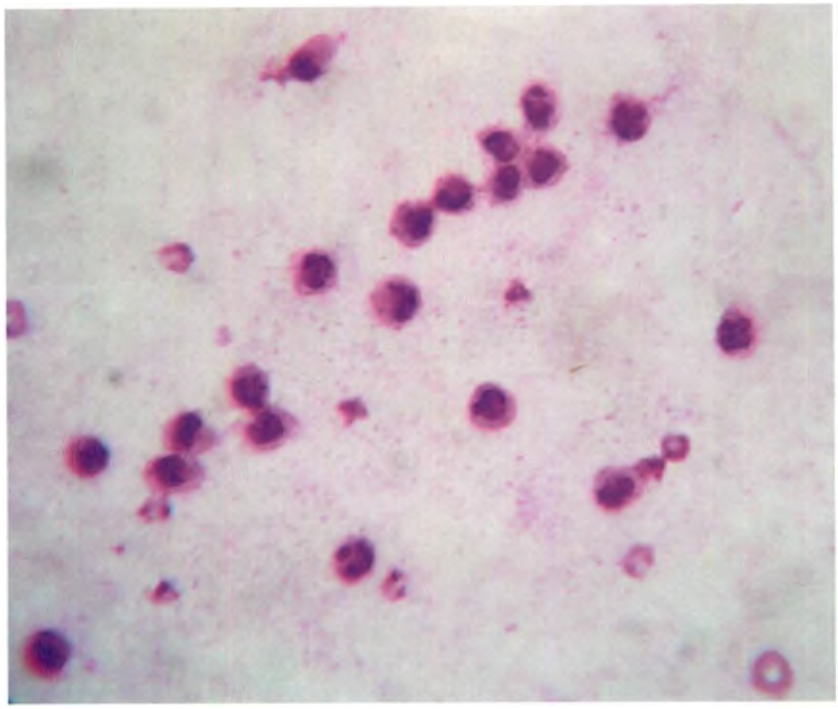

Fig.25. Synovial fluid smear examination (10x)

Fig.26. Characteristic pink colonies of E.coli

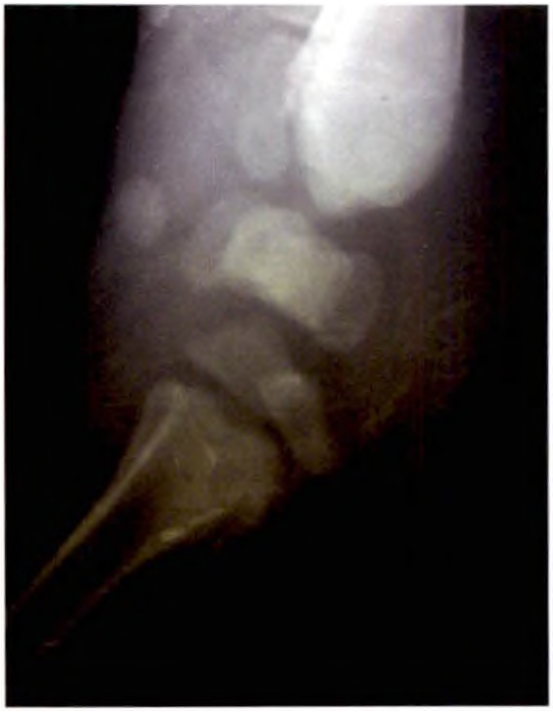

Fig.27. Skiagram of the knee joint of a calf (II_1)-before treatment

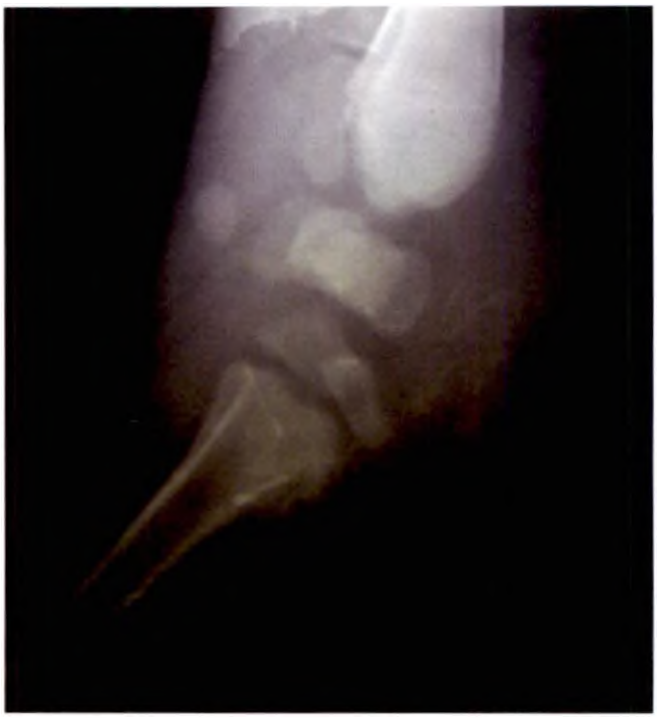

Fig.28. Skiagram of the knee joint of a calf (II_1)-15th day of treatment

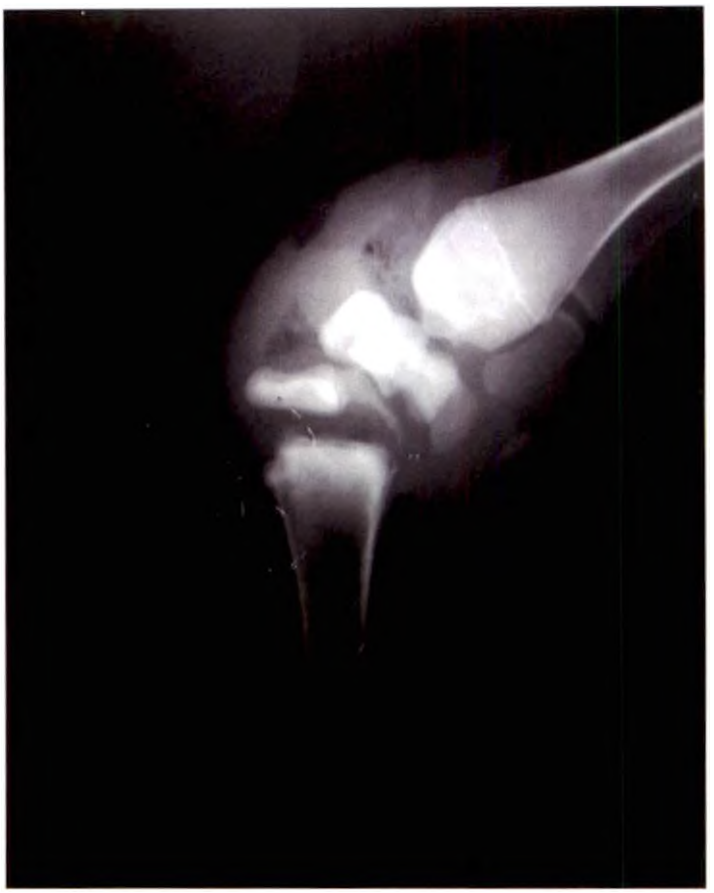

Fig.29. Skiagram of the knee joint of a calf (II_3)-before treatment

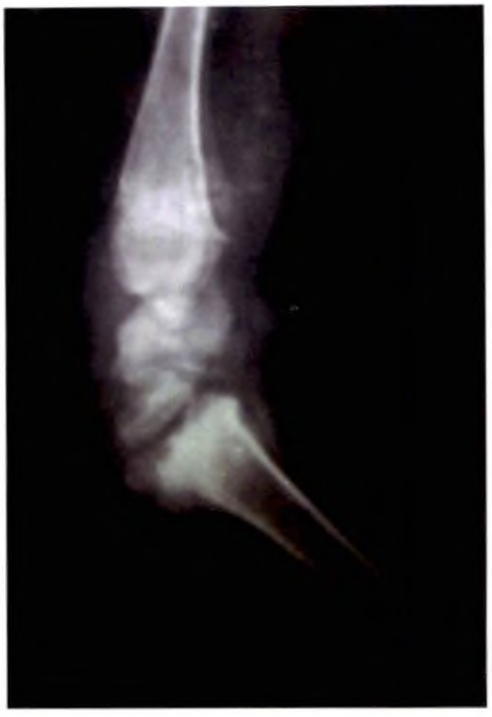

Fig.30. Skiagram of the knee joint of a calf (II_6)-before treatment

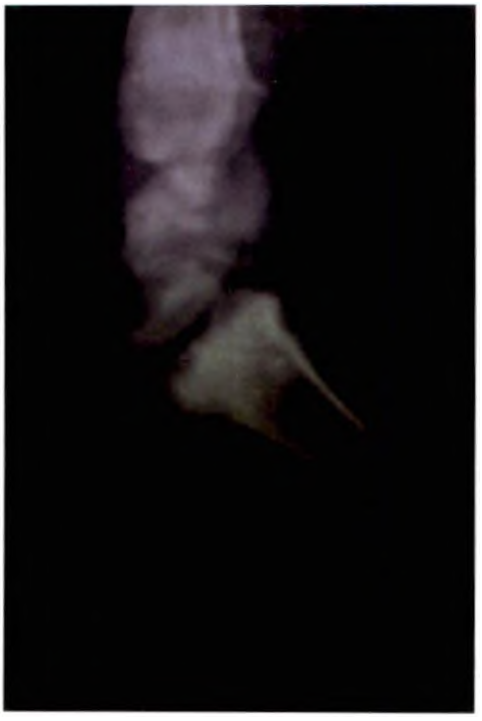

Fig.31. Skiagram of the knee joint of a calf (II_6)-15th day of treatment

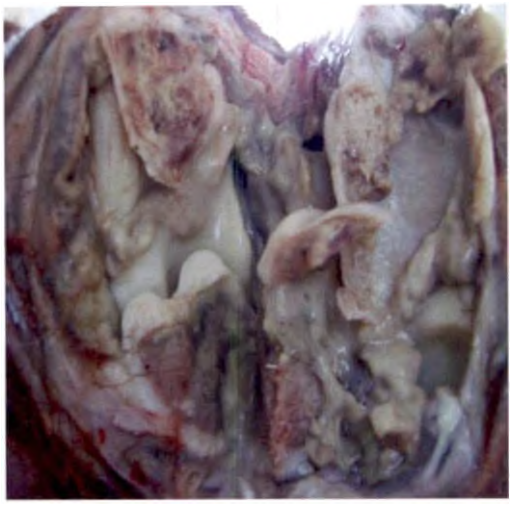

Fig-32. Severe degeneration of articular cartilage-calf II_3

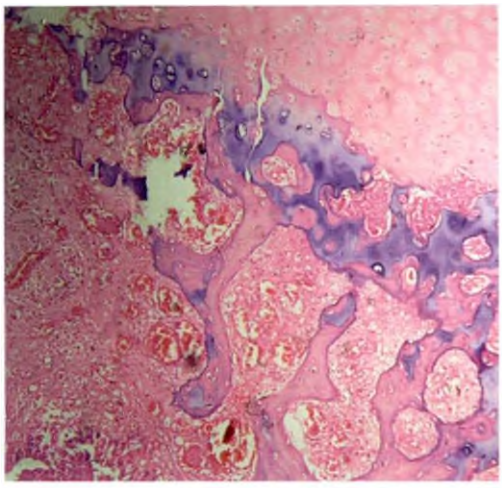

Fig.33. Thick hyalinised tissue with areas of haemmorrhage and congested blood vessels (10x)

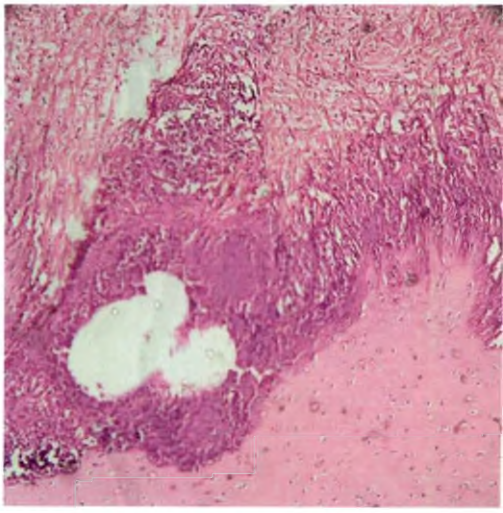

Fig.34. Dense infiltration of inflammatory cells (10x)

Discussion

5. DISCUSSION

Arthritis refers to inflammation of joint and in calves its consequences have many economic implications. The losses occur in the form of death or culling of affected animals, expensive treatment and poor production performance. Arthritis in calves requires careful evaluation to arrive at an early diagnosis, which is essential to adopt an effective surgical management.

The present study was carried out in twelve calves affected with arthritis aged below six months belonging to different breeds of either sex, presented to the College Veterinary Hospital at Mannuthy and University Veterinary Hospital at Kokkalai. Details about signalment and anamnesis were collected. All the calves were subjected to detailed clinical, physiological, haematological and serum biochemical evaluation. Radiographic and synovial fluid evaluations of the affected joint(s) were also carried out. Based on the nature of synovia, whether it was non-purulent or purulent, the calves were divided into Group I and II respectively, consisting of six calves each. Animals in Group I were treated by intra-articular antimicrobial therapy and in Group II by lavaging the affected joint with ten per cent dimethyl sulfoxide solution (DMSO) using 'through-and-through' needle technique with supplementation of parentral antibiotic therapy to the animals of both the groups.

5.1. SIGNALMENT

5.1.1. Age

The age of affected calves ranged from five days to two months in Group I and five days to one month in Group II. According to Wooldridge (1934) and Singh and Tayal (1993) infectious arthritis was commonly encountered in young stock. Chawla *et al.* (1989a) reported that septic arthritis was a common clinical problem in cattle and buffaloes, with higher incidence in young calves.

5.1.2. Breed

The breeds affected were Holstein- Friesian cross and Jersey cross bred with three animals each in Group I and one and five animals respectively in Group II.

5.1.3. Sex

The male and female calves were three each in Group I and one and five respectively in Group II.

5.1.4. Joint(s) affected

The knee joint was affected in both the groups and was unilateral in Group I with the right knee joint in four calves and the left in two calves. Bilateral affection of right and left knee joints were observed in two calves of Group II and unilateral affection of right knee joint in three calves and the left knee joint in one calf. According to the report of Jackson (1999), the most commonly affected joints by septic arthritis in calves were carpus, hock and stifle.

5.2. ANAMNESIS

From the history of affected calves, it was revealed that four calves in Group I had the history of umbilical infection and the umbilical cords were torn naturally at the time of birth and the stumps were left untreated. Radostits *et al.* (1985) opined that navel ill, especially extension of infection into the abdominal cavity, if not diagnosed or treated promptly, might lead to complications like polyarthritis, meningitis, cataract and liver damage in calves. Prasanna (2003) recorded the incidence of suppurative arthritis of both knee joints in one calf which had the history of extra-abdominal umbilical infection. But no history of umbilical infection in any of the calves in Group II was reported. Wooldridge (1934) recorded that septic arthritis can occur in calves occasionally before birth

by intra-uterine route. Two calves in Group I was treated previously for umbilical infection by surgical drainage, local dressing and antibiotic therapy with streptomycin- penicillin combination. One calf in Group II was treated previously with sulpha bolus. Two calves in Group I and one in Group II were not sufficiently fed with colostrum. The duration of illness ranged from three days to one week in Group I calves and two days to one week in Group II calves. According to Wooldridge (1934), the symptoms of arthritis in calves, foals and lambs would occur in about a week or ten days after birth upto as long as six months or even longer. Pratap *et al.* (1977) reported that physical symptoms developed in the joints of calves, which were experimentally inoculated with pathogenic organisms, commenced 48-72 hours post- inoculation. It was also reported that anamnesis proved to be an excellent means for proper diagnosis of arthritis.

5.3. CLINICAL OBSERVATIONS

Subjective monitoring of the affected joint was performed in the calves of both Group I and II. The affected joints were swollen, warmth to touch and evinced pain on palpation in all calves of both the groups at the time of presentation to the hospital. No joint exudation were coming out and the skin was remaining intact in all the calves of Group I. But open joints were present in all the calves of Group II. Deviation from normal posture and gait was evidenced in all calves of both the groups at the time of presentation to the hospital. Pointing of toe was noticed in the affected limb in three calves of Group I and in Group II, it was present only in two calves. In all the calves of Group I, the affected limb could be flexed and evidenced pain while flexing. In Group II, flexing of affected knee joint was difficult in three calves and in the other three calves, flexing was possible. There was protrusion of necrotic tissues through the knee joint opening and the animal was recumbent at the time of presentation to the hospital.

The joint swelling and pus was reduced by the fifth day of observation in two calves of Group I and II and further improved by the fifteenth day in two

calves of Group II. In three calves of Group I, the swelling and warmth of joint was almost absent by the fifteenth day. But in one calf of the group, the swelling and warmth persisted till two months. Pointing of toe in the three calves of Group I was absent by the fifteenth day of observation after treatment. In Group II, pointing of toe was persistent till the fifteenth day of observation in one among the two calves and the other one succumbed to death by the seventh day of observation after joint lavage was performed. Pain upon palpation of affected joint was absent by the fifth day after treatment in one calf and in the rest of the calves by the fifteenth day in Group I. In Group II, improvement was noticed regarding pain upon palpation of affected joint by the fifteenth day. The observations of Pratap *et al.* (1977), Gustafson (1993), Jackson (1999) and Bennet (2005) revealed that in animals affected with septic arthritis, the infected joints were warm, swollen and painful on manipulation. Lameness was often the predominant clinical sign. Several lameness scoring systems for both large and small animals were available, of which the one designed by Anderson and Desrochers (2004) was followed in this study.

5.4. PHYSIOLOGICAL OBSERVATIONS

The mean rectal temperature, pulse rate and respiratory rate were found within normal range in both the groups, throughout the period of study. Van Pelt *et al.* (1968) however, stated that in infectious arthritis of calves attributable to metastasis of bacteria, pyrexia was a general manifestation. The mean rate of pulse and respiration before treatment and post treatment periods in this study indicated that these factors were of no diagnostic relevance, which was in accordance with the observation made by Pratap *et al.* (1977).

5.5. HAEMATOLOGICAL OBSERVATIONS

5.5.1. Haemoglobin concentration: The haemoglobin concentration was within the normal range before treatment and during the rest of the period of study in

both the groups. Pratap *et al.* (1977) reported that the mean haemoglobin value was insignificant in the diagnosis of infectious arthritis in calves in early stages.

5.5.2. Volume of packed red cells (VPRC): The volume of packed red cells was also within the normal range in both the groups throughout the period of study. According to Pratap *et al.* (1977) the mean volume of packed red cells was insignificant in the diagnosis of infectious arthritis in calves in the early stages. As per Benjamin (1985) the haemoglobin and the volume of packed red cells were high at birth in cattle, but were reduced as soon as the calf begins to nurse. With the calf increasing in size and the input of iron low, these values might continue to decline for several weeks.

5.5.3 Total leukocyte count (TLC): Various factors might contribute to physiological leukocytosis, such as time of day, a meal, exercise, stress conditions etc. Some species of animals might be born with an increased number of leukocytes. But in the new born calf the number was similar to that found in adult Swenson (1993). The mean total leukocyte count was higher before treatment in both the groups and in Group I, it was within the normal range from the fifth day and throughout the period of observation. In Group II, it was within the normal range only by the fifteenth day of observation. The high mean value of TLC before starting treatment could be explained by the fact that leukocytosis was the result of the stress of infection.

5.5.4. Differential leukocyte count (DLC): The neutrophil count was higher and the lymphocyte count was lower before treatment and almost reached towards normal range by the fifteenth day of observation in both the groups. A similar type of observation was recorded by Pratap *et al.* (1977) in septic arthritis of calves due to *Escherichia coli.* Van Pelt *et al.* (1966) reported that mean proportion of segmented neutrophil was significantly increased while the mean proportion of lymphocyte was significantly less in the blood of arthritic calves. Monocytes were not obtained in any of the calves in Group I. The monocyte

value was lower than the normal range throughout the observation period in Group II and basophils were absent.

5.6. SERUM BIOCHEMICAL OBSERVATIONS

5.6.1. Glucose: The new born ruminants have blood glucose values similar to those of non-ruminant mammals. These values decrease sharply during the first few weeks of life and then decrease more slowly until adult values are reached Beitz (1993). The serum glucose level was within the normal range throughout the period of observation in both the groups. The serum glucose estimation is important, but only along with synovial fluid sugar estimation, as according to Van Pelt and Conner (1963b) blood or plasma sugar to synovial fluid sugar difference was important in the interpretation and evaluation of intra-articular metabolism and various arthritis conditions.

5.6.2. Alkaline phosphatase: Elevations of serum alkaline phosphatase were observed in normal growing animals or in adult animals with increased osteoblastic activity. The enzyme's activity might also be elevated in acute or chronic liver diseases Tennant (1985). The mean alkaline phosphatase level was within the normal range in this study as per Allcroft and Folley (1941), throughout the observation period, even though a slight reduction within the normal range was observed on the fifth and fifteenth day after treatment in Group I and II.

5.7. SYNOVIAL FLUID EVALUATION

5.7.1. Physical evaluation: The volume, colour, mucin clot quality, odour and time of clotting were assessed. The mean volume collected was slightly higher in both the groups. The colour of the samples in Group I was slightly yellowish and in Group II, it was turbid yellow with some flocculent materials. The mucin clot quality was normal in Group I calves except in two animals, which graded as fair. Fair, poor and very poor mucin clot qualities were obtained in two samples each

in Group II. All collected samples were odourless in Group I and two samples had a pungent odour in Group II. There was clotting of synovial fluid sample within ten minutes in one sample and the rest of the samples did not clot within one hour in Group I. Clotting was observed within five minutes in two samples, within ten minutes, seven minutes, twenty minutes and one hour in one sample each of Group II. According to Van Pelt and Langham (1968) increased volume of synovial effusion from infected joints was a constant finding, varying from one joint to another, relative to individual joint size. The gross appearance of synovial effusions varied with the duration of infection and virulence of microorganism for joint tissues. Most synovial effusions were turbid and yellow, with various amounts of flocculent material. Yellow or amber synovial effusions were indicative of high bilirubin content, which varied in direct proportion to the respective colour. Infectious synovial effusions clotted rapidly in the absence of added anticoagulant. Van Pelt (1975) reported that opacity and flocculation in synovial fluid could be attributed to the presence of cartilaginous fragments and fibrils. Chawla *et al.* (1989a) observed that the synovial fluid became turbid, yellow and opaque with varying amounts of flocculent materials.

5.7.2. Cytological evaluation: Differential leukocyte count was performed. Only neutrophils and lymphocytes were obtained. The mean neutrophil count per cent was very high in both the groups. Eosinophils were rare and basophils were hard to find. Madison *et al.* (1991) opined cytologic evaluation of synovial fluid samples as the probable single most useful test in evaluating a joint with suspected infection. According to Van Pelt and Langham (1968) exudative synovial effusions were characterised by high total leukocytic counts, especially increased absolute numbers of neutrophils. Examination of synovial fluid smears stained with Wright's stain provided a rapid means of differentiating polymorpho-nuclear neutrophils, from the various mononuclear leukocytes. It should be pointed out that closer attention to morphologic details and staining reaction was required in order to accurately evaluate and differentiate synovial fluid lymphocytes, monocytes and macrophages. Eosinophils were rarely

observed in the stained smears and basophilic leukocytes were never observed in stained smears of synovial fluid (Van Pelt and Conner, 1963a). In view of such findings Warren *et al.* (1935) opined that, cells in synovial fluid were not to be considered as a true reflection of the cellular constituents in peripheral blood.

5.7.3. Biochemical evaluation: The mean glucose levels were within the normal range in Group I and lower in Group II. According to Van Pelt and Conner (1963b), the concentration of sugar in synovial fluid was approximately equal to that in blood in normal cattle and the difference between the two values had diagnostic importance as per Van Pelt and Langham (1968) and accordingly it was reported that the synovial fluid sugar to blood sugar ratio of 0.5:1.0, for cattle affected with infectious arthritis was considerably altered in contrast to the 1:1 ratio reported for normal cattle by Van Pelt and Conner (1963b). It was opined by Van Pelt and Langham (1968) that the reduced sugar supply, combined with greater utilization by inflamed or proliferated synovial membranes and an increased number of neutrophils and microorganisms, might have resulted in the pronounced lowering of the synovial fluid sugar level. It was also observed that there was a close relationship between the severity of infectious arthritis and an increase in the absolute numbers of neutrophils in conjunction with low levels of synovial fluid sugar.

The mean alkaline phosphatase level was observed to be higher than the levels obtained in serum in both the groups. This was in accordance with the finding of Van Pelt and Langham (1968) and opined that articular cartilages and elevated numbers of synovial fluid neutrophils as the primary source of alkaline phosphatase in synovial effusions from joints of cattle affected with infectious arthritis. According to Bennet (1985) there was circumstantial evidence that the alkaline phosphatase of osteoblasts might be involved in bone calcification. Hence it can be considered that the complications occurring in arthritis such as ankylosis might be correlated with the higher levels of alkaline phosphatase in synovial effusions.

5.7.4. Culture and sensitivity test: In Group I, no organisms could be isolated from the synovial fluid samples. But one isolate of *Escherichia coli* could be obtained from the umbilical pus in one calf of the same group and gentamicin was the most sensitive antibiotic. Verschooten *et al.* (1974) opined that culture and sensitivity results could not always be correlated with clinical responses to treatment. According to Bertone *et al.* (1987b) negative culture results from synovial fluid may be due to primary localization of bacteria in synovial membrane. Clements *et al.* (2005) reported that a negative result from a bacterial culture of synovial fluid, from clinically infected or experimentally infected joints could support the assertion that a positive bacterial culture might not be a necessity for the diagnosis of bacterial infective arthritis. According to May (2005) prior use of antibacterial agents, even as a single dose could reduce the chances of a successful culture from synovial fluid. Two isolates of *Escherichia coli* were obtained, among the six samples of synovial fluid taken from Group II and characteristic pink colonies were obtained on Mc Conkey agar plates. Orsini (1996) reported *Streptococcus spp.* and *Escherichia coli* as the most commonly isolated organisms in young calves affected with septic arthritis, followed by *Actinomyces pyogenes*, *Salmonella spp.*, *Mycoplasma bovis* and *Haemophilus somnus*. Sulphadiazine was the most sensitive antibiotic in one isolate and gentamicin in the other one.

5.8. RADIOGRAPHIC EVALUATION

Characteristic radiographic abnormalities were not present in Group I, of which only soft tissue swelling was observed as there was an increase in soft tissue density. Soft tissue swelling in and around the joint was the only radiographic evidence in early stages of joint infection (May, 2005). Other identified lesions in the present study were widened joint space, intra-articular gas shadow, subchondral osteolysis and blurring of normal bone outline in Group II calves. Similar lesions were observed by Ramanathan (2007). According to Moulvi *et al.* (2001) radiographs of septic arthritis of carpal joints in calves

showed considerable increase in soft tissue density and slight increase in joint space. It was also reported that these changes did not show much improvement even by the 40th day radiograph.

5.9. HISTOPATHOLOGICAL FINDINGS

Histopathology was performed in two calves which succumbed during the observation period. Macroscopic examination revealed swollen joints and upon opening discharge of pus with degeneration of articular cartilage was noticed. Microscopic examination revealed thick hyalinised fibrocollagenous tissue, areas of haemorrhage with many congested blood vessels. Some areas appeared like abscess wall of which was formed by inflammatory granulation tissue. One section showed bony tissue with degenerating bony trabeculae. Periosteum and adjacent tissues showed extensive necrosis and abscess formation. Similar observations were made by Van Pelt *et al.* (1966) and reported that the histopathological lesions varied with the virulence of the microorganism for joint tissue and duration of the disease.

5.10. OTHER OBSERVATIONS, IF ANY

Moulvi *et al.* (2002) recommended single dose intra-articular administration of gentamicin for treatment of septic arthritis in calves. Out of the six calves treated in Group I three (I_4, I_5 and I_6) fully recovered with single intra-articular therapy, another three (I_1, I_2 and I_3) with repeated intra-articular therapy on the fifth day. Lloyd *et al.* (1988a) reported that the mean synovial fluid concentration of gentamicin remained well above the minimal inhibitory concentration of gentamicin for many bacterial pathogens. Single 'through-and-through' needle lavage using ten per cent DMSO proved to be effective in one calf of Group II. In other two animals of the same group, the lavage yielded good result by repeating it on the fifth day. Two animals of Group II succumbed, one calf after one month and the other within seven days after the initiation of treatment. Ankylosis of the affected joint which was present before treatment in

two calves of Group II could not be resolved by the lavage technique using ten per cent DMSO. Application of elastic crepe bandage for compression proved to be effective in providing adequate immobilization of the affected joint and thereby reducing the inflammatory reaction. DMSO has definitely got anti-inflammatory property as the pus of the affected joints considerably reduced after treatment in four calves of Group II and the open joints showed healing and epithelialisation indicating that, it is a good counter irritant also. These properties of DMSO might be due to the ability of DMSO to prevent the depolymerisation of hyaluronic acid by oxygen derived free radicals, free radical scavenging property and through direct suppression of prostaglandin production, as reported by Fox (1983) and Alsup and DeBowes (1984).

Summary

6. SUMMARY

The present study was carried out in twelve calves affected with arthritis aged below six months belonging to different breeds of either sex, presented to the College Veterinary Hospital at Mannuthy and University Veterinary Hospital at Kokkalai. Details about signalment and anamnesis were collected. All the calves were subjected to detailed clinical, physiological, haematological and serum biochemical evaluation including the radiographic and synovial fluid evaluation of the affected joint(s). Based on the nature of synovia, whether it was non-purulent or purulent, the calves were divided into Group I and II respectively, each consisting of six calves and were serially numbered from 1 to 6. Animals in Group I were treated by intra-articular antimicrobial therapy and in Group II by lavaging the affected joint with ten per cent dimethyl sulfoxide (DMSO) using 'through-and-through' needle technique with supplementation of parentral antibiotic therapy to the animals of both the groups.

The calves affected were Holstein- Friesian cross and Jersey cross breeds with three animals each in Group I and one and five animals respectively in Group II. The male and female calves were three each in Group I and one and five respectively in Group II. The age of affected calves ranged from five days to two months in Group I and five days to one month in Group II. The knee joint was affected in both the groups and was unilateral in Group I with the right knee joint in four calves and the left in two calves. Bilateral affection of right and left knee joints were observed in two calves of Group II and unilateral affection of right knee joint in three calves and the left knee joint in one calf. Four calves in Group I had the history of umbilical infection and the umbilical cords were torn naturally at the time of birth and the stumps were left untreated. But no history of umbilical infection in any of the calves in Group II. Two calves in Group I was treated previously for umbilical infection by surgical drainage, local dressing and antibiotic therapy with streptomycin- penicillin combination. One calf in Group II was treated for joint swelling previously with sulpha bolus. Two calves in

Group I and one in Group II were not sufficiently fed with colostrum. The duration of illness ranged from three days to one week in calves of Group I and two days to one week in Group II.

Subjective monitoring of the affected joint was performed in the calves of both Group I and II. The affected joints were swollen, warm to touch and evidenced pain upon palpation in all calves of both the groups at the time of presentation to the hospital. Joint exudations were not coming out and the skin was remaining intact in all the calves of Group I. But open joints were present in all the calves of Group II. Deviation from normal posture and gait was evidenced in all calves. Pointing of toe was noticed in the affected limb in three calves of Group I and in Group II, it was present only in two calves. In all the calves of Group I, the affected limb could be flexed and evidenced pain while flexing. In Group II, flexing of affected knee joint was difficult in three calves and in the other three calves, flexing was possible. In two calves of Group II there was protrusion of necrotic tissues through the knee joint opening and the calves were recumbent.

Following treatment, the joint swelling was reduced by the fifth day of observation in two calves each of Group I and II and further improved by the fifteenth day in two calves of Group II. In three calves of Group I, the swelling and warmth of joint was almost absent by the fifteenth day. But in one calf of the group, the swelling and warmth persisted till two months and in Group II, the swelling persisted throughout the period of observation in four calves. Pointing of toe in the three calves of Group I was absent by the fifteenth day of observation after treatment. In Group II, pointing of toe was persistent till the fifteenth day of observation in one among the two calves and the other one succumbed to death by the seventh day of observation after joint lavage was performed. Pain upon palpation of affected joint was absent by the fifth day after treatment in one calf and in the rest of the calves by the fifteenth day in Group I. In Group II,

improvement was noticed regarding pain upon palpation of affected joint by the fifteenth day.

The mean rectal temperature, pulse rate and respiratory rate were found within normal range in both the groups, throughout the period of study.

The haemoglobin concentration and the volume of packed red cells were within the normal range before treatment and during the rest of the period of observation in both the groups. The mean total leukocyte count was higher before treatment in both the groups and in Group I it was within the normal range from the fifth day and throughout the period of observation. In Group II, it was within the normal range only by the fifteenth day of observation. The neutrophil count was higher and the lymphocyte count was lower before treatment and almost reached towards normal range by the fifteenth day of observation in both the groups. Monocytes were not obtained in any of the calves in Group I. The monocyte value was lower than the normal range throughout the observation period in Group II. Basophils were absent in both the groups.

The serum glucose level was within the normal range throughout the period of observation in both the groups. The mean serum alkaline phosphatase level was within the normal range in this study throughout the observation period, even though a slight reduction within the normal range was observed on the fifth and fifteenth day after treatment in Group I and II.

The colour, mucin clot quality, odour and time of clotting of synovial fluid were assessed before initiation of treatment. The colour of the samples in Group I was slightly yellowish and in Group II, it was turbid yellow with some flocculent materials. The mucin clot quality was normal in Group I calves except in two animals, which graded as fair. In Group II, fair, poor and very poor mucin clot qualities were obtained in two samples each. All collected samples were odourless in Group I and two samples had a pungent odour in Group II. There was clotting of synovial fluid sample within ten minutes in one sample and the

rest of the samples did not clot within one hour in Group I. Clotting was observed within five minutes in two samples, within ten minutes, seven minutes, twenty minutes and one hour in one sample each of Group II. Differential leukocyte count was performed. Only neutrophils and lymphocytes were obtained. The mean neutrophil count per cent was very high in both the groups. Eosinophils were rare and basophils were hard to find. The mean glucose levels were within the normal range in Group I and lower in Group II. But the mean alkaline phosphatase level was observed to be higher than the levels obtained in serum in both the groups.

In Group I, no organisms could be isolated from the synovial fluid samples. But one isolate of *Escherichia coli* was obtained from the umbilical pus in one calf of the same group and it was most sensitive to the antibiotic gentamicin. Two isolates of *Escherichia coli* were obtained, among the six samples of synovial fluid taken from Group II. Sulphadiazine was the sensitive antibiotic in one isolate and gentamicin in the other one.

In Group I, the affected joints were without any characteristic radiographic abnormalities except for the increase in soft tissue density due to soft tissue swelling. Whereas in Group II lesions identified were widened joint space, intra-articular gas shadow, subchondral osteolysis and blurring of normal bone outline.

All the calves of Group I, in which intra-articular antimicrobial therapy was performed, showed good response. But in one calf of the same group the joint swelling persisted even on the fifteenth day of observation and it was resolved completely only by two months. Intra-articular therapy was repeated on the fifth day in three calves of Group I and the results were good. Single 'through-and-through' needle lavage using ten per cent DMSO proved to be effective in one calf of Group II. In other two animals of the same group, the lavage yielded good result by repeating it on the fifth day. Two animals of Group II succumbed, one calf after one month and the other within seven days after the

initiation of treatment. Ankylosis of the affected joint which was present before treatment in two calves of Group II could not be resolved by the lavage technique. Application of elastic crepe bandage for compression proved to be effective in providing adequate immobilization of the affected joint and thereby reducing the inflammatory reaction. The pus of the affected joints considerably reduced after the lavage with DMSO in four calves of Group II and the open joints showed healing and epithelialisation.

Histopathology of affected joint was performed in two calves which succumbed during the observation period. Macroscopic examination revealed considerable swelling of joint with pus and degeneration of articular cartilage. Microscopic examination revealed thick hyalinised fibrocollagenous tissue, areas of haemorrhage with many congested blood vessels. Some areas appeared like abscess wall of which was formed by inflammatory granulation tissue. One section showed bony tissue with degenerating bony trabeculae. Periosteum and adjacent tissues showed extensive necrosis and abscess formation.

From the present study, it could be concluded that:

1. Knee joint was the most susceptible joint for arthritis in calves which may be either unilateral or bilateral.

2. Umbilical infection occurring during birth could lead to arthritis in calves.

3. Physical symptoms such as swelling, pain, warmth of the affected joint, inability to flex the joint and lameness are very much characteristic and help in the diagnosis of arthritis.

4. Physiological, haematological, serum biochemical, microbiological and radiographic evaluation may not be effective in diagnosing the initial stages of arthritis of calves whereas, synovial fluid evaluation proved to be a good tool in assessing the severity and prognosis of arthritis

5. Intra-articular antimicrobial therapy with supplementation of parentral antibiotics in arthritis with non-purulent synovia gave promising results.

6. 'Through-and-through'lavage using ten per cent dimethyl sulfoxide (DMSO) in arthritis with purulent synovia gave good results if performed in the initial stages.

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INTRA-ARTICULAR ANTIMICROBIAL THERAPY AND LAVAGE FOR THE MANAGEMENT OF ARTHRITIS IN CALVES

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ABSTRACT

The present study was carried out in twelve calves affected with arthritis under six months of age belonging to either sex, presented to the College Veterinary Hospitals at Mannuthy and Kokkalai. Based on the nature of synovia, whether it was non-purulent or purulent, the calves were divided into two groups *viz.* Group I and II, each consisting of six calves and were serially numbered from 1 to 6. Animals in Group I were treated by intra-articular antimicrobial therapy and in Group II by lavaging the affected joint with ten per cent DMSO using 'through-and-through' needle technique with supplementation of parentral antibiotic therapy to the animals of both the groups.

Both male and female, Holstein-Friesian cross and Jersey cross bred calves with age group ranging from five days to two months were presented. In all the calves, the knee joint was affected, either unilaterally or bilaterally. Out of the 12 calves, four had the history of umbilical infection and in which the umbilical cords were torn naturally at the time of birth and the stumps were left untreated. The duration of illness ranged from two days to one week.

The affected joints were swollen, warm to touch and evidenced pain on palpation with difficulty in flexing the joint. In a few calves purulent exudation was coming out. In Group I, all the calves recovered within five days of treatment except one which also got recovered, but only after two months. In Group II, promising recovery was observed in two calves within fifteen days of observation and in one recovery with persistent ankylosis, while two calves succumbed during the observation period.

The mean rectal temperature, pulse rate, respiratory rate, haemoglobin concentration and VPRC were found within normal range in both the groups, throughout the period of study.

The mean total leukocyte count was higher before treatment in both the groups and it was within the normal range from the fifth and fifteenth day of observation in Group I and II respectively. The neutrophil count was higher and

the lymphocyte count was lower before treatment and almost reached towards normal range by the fifteenth day of observation in both the groups.

The serum glucose and alkaline phosphatase levels were within the normal range throughout the period of observation in both the groups.

In Group I, the synovia was yellowish, odourless and with normal to fair mucin clot quality and prolonged clotting time. In Group II, it was turbid yellow with flocculent materials and pungent odour (in two cases) with fair to very poor mucin clot quality and quickened clotting time. The mean glucose levels were within the normal range in Group I and lower in Group II. But the mean alkaline phosphatase level was observed to be higher than the levels obtained in serum in both the groups.

Escherichia coli was the major organism isolated from two samples of synovial fluid among the twelve samples collected and gentamicin was sensitive in one isolate, while Sulphadiazine in the other one.

In Group I, the affected joints were without any characteristic radiographic abnormalities except for the increase in soft tissue density due to soft tissue swelling. Whereas in Group II lesions identified were widened joint space, intra-articular gas shadow, subchondral osteolysis and blurring of normal bone outline. There were not many variations in the fifteenth day post-treatment radiographs.

Histopathology of affected joint was performed in two calves which succumbed during the observation period. Macroscopic examination revealed considerable swelling of joint with pus and degeneration of articular cartilage. Microscopic examination revealed thick hyalinised fibrocollagenous tissue, areas of haemorrhage with many congested blood vessels. Some areas appeared like abscess wall of which was formed by inflammatory granulation tissue. One section showed bony tissue with degenerating bony trabeculae. Periosteum and adjacent tissues showed extensive necrosis and abscess formation.