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Study of synovial fluid characteristics in calves affected with septic arthritis

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Abstract
This study aims to evaluate the changes in synovial fluid and compare treatment protocols for calves affected by septic arthritis. Among 2,412 bovine cases examined, 36 were diagnosed with septic arthritis, representing an incidence rate of 1.49%. Of these, twelve calves were selected and randomly assigned into two groups of six. Each group received an intra-articular chitosan-based drug delivery system, one impregnated with ceftiofur sodium and the other group with honey. At the time of presentation, most synovial fluid samples were purulent, opaque and turbid, containing suspended fibrinous material. By day 14, improvements were observed in both groups, with the synovial fluid becoming noticeably clearer and more viscous. Parameters such as total protein, total white blood cell (WBC) count, polymorphonuclear (PMN) cell count and synovial fluid or joint discharge were significantly elevated at presentation but returned to normal levels by day 28 in both treatment groups.

Keywords: Sepsis, arthritis, calves, synovial fluid, joints

1. Introduction
Septic arthritis is a "frequently encountered condition in neonatal calves, associated with considerable economic losses due to elevated morbidity and mortality (Ibrahim, 2019) [1]. Beyond calves, it also represents a significant health concern in cattle (Steiner *et al.*, 1999) [2] and equines (Lapointe *et al.*, 1992; Steel *et al.*, 1999; Meijer *et al.*, 2000) [3-5]. Management of the disease is challenging, often necessitating prolonged treatment and substantial veterinary costs. Due to limited therapeutic efficacy, a large proportion of affected animals are culled or slaughtered annually (Wichtel *et al*., 2003; Desrochers, 2004; Goodarzi *et al*., 2015) [6-8]. Septic arthritis is recognized as a major cause of lameness in calves during the first eight weeks of life, typically arising from bacterial invasion of the joint. If not diagnosed and treated promptly, the infection can result in irreversible joint destruction (Jackson, 1999) [9]. Characteristic alterations in synovial fluid have been described in septic arthritis. Martens *et al.* (1986) [10] reported that infected synovial fluid is typically turbid, amber to hemorrhagic in color, and exhibits reduced viscosity. Similarly, Moulvi *et al.* (1993) [11] noted that while normal synovial fluid is colorless to straw-colored, clear and devoid of flocculent material, septic synovial fluid is pale to dark yellow, turbid, and contains visible flocculations. Biochemical and cytological changes have also been well documented. St-Jean (1993) [12] observed increased synovial fluid volume, yellow to brown discoloration, flocculent material, reduced viscosity, elevated protein concentration (>2 g/100 ml), and leukocyte counts exceeding 30,000 cells/µl, with neutrophils accounting for >90%. Kumar and Singh (1995) [13] further reported increased protein, leukocyte counts, alkaline phosphatase activity, and synovial fluid volume, along with decreased pH, viscosity, mucin clot quality, and glucose concentration in calves with *Staphylococcus aureus*-induced arthritis. Altintas *et al.* (2010) [14] highlighted that protein levels >2 mg/ml in synovial fluid indicate pathological changes, while glucose concentrations are reduced by approximately 55% compared to healthy calves."

2. Materials and Methods

The present study was conducted on clinical cases of calves affected with septic arthritis and presented to the Veterinary College, Hebbal (Bangalore), the College of Veterinary Science, Tirupati and the Veterinary Hospital, Visakhapatnam. Out of 36 affected calves, 12 were selected and were randomly divided into two experimental groups comprising six animals each. Calves in Group I were treated with intra-articular administration of chitosan impregnated with ceftiofur sodium (100 mg), whereas those in Group II received intra-articular honey (5 ml). Synovial fluid samples were collected and subjected to physicochemical and cytological analysis, including estimation of pH, total protein, total leukocyte count and proportion of polymorphonuclear cells. The data generated were statistically analyzed using one-way analysis of variance (ANOVA) and independent two-sample t-tests with the aid of SPSS version 20.

2.1 Analysis of joint discharges/fluid analysis:

All affected joints were clipped, shaved and thoroughly prepared using 7.5% povidone-iodine scrub followed by cleansing with surgical spirit to ensure asepsis. In cases where the joint was open, a sterile cotton-tipped swab was carefully introduced into the joint cavity to obtain a sample (Fig. 1). For closed joints, arthrocentesis was carried out under aseptic conditions using an 18 G needle. In severely affected cases, arthrotomy was performed after infiltration of 5 ml of 2% lignocaine solution at the selected site. Throughout both arthrocentesis and arthrotomy, synovial fluid samples were collected under strict aseptic precautions to avoid secondary contamination of the joint (Fig. 2).

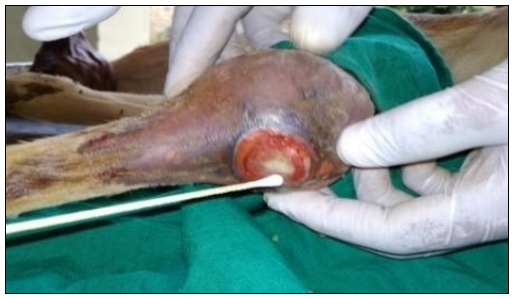

Fig 1: Collection of synovial sample by using a sterile cotton tipped swab

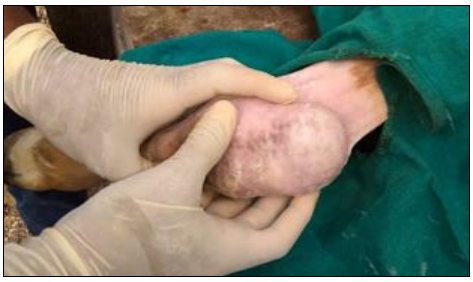

Fig 2: Exposure of swollen joint for arthrocentesis /arthrotomy

2.1.1 Synovial fluid colour and consistency

Samples were evaluated for characteristics such as (colorless, hemorrhagic, or yellowish), gross appearance (clear, turbid, or purulent) and the presence of flocculent particulate matter.

2.1.2 Synovial fluid /discharge pH

The pH of synovial fluid/discharges collected from both groups was determined using pH indicator strips.

2.1.3 Synovial fluid /discharge total protein

Total protein (g/dl) in synovial fluid/joint discharges was quantified using an automated biochemistry analyzer (Mindray BS-120, China)

2.1.4 Synovial fluid /discharge total WBC count

Synovial fluid was diluted 1:2 with saline, loaded into a hemocytometer, and total leukocytes were enumerated under a light microscope.

2.1.5 Synovial fluid /discharge PMN cells

Thin smears of synovial fluid were prepared, air-dried, stained with Leishman and examined under a light microscope.

3. Results

Thirty-six cases of calves with septic arthritis including 48 joints were presented. Out of which 41 were carpal joints (85.41%), five were tarsal joints (10.4%) and two were fetlock joints (4.1%).

3.1 Synovial fluid/ Joint discharge analysis

3.1.1 Synovial fluid colour and consistency

The color and consistency of joint discharges in both groups varied from colorless to yellow or reddish from the day of presentation up to day 28. In most cases, the discharge appeared yellowish, ranging from watery yellow fluid containing flocculent material to thick, creamy, or cheesy yellow exudate. By day 14 following initiation of treatment, the synovial fluid in both groups showed marked improvement, becoming relatively clear and viscous. The gross characteristics of joint discharges/synovial fluid collected from different joints are summarized in Table 1 and illustrated in Figure 3.

Table 1: Synovial fluid/ joint discharge appearance in calves with septic arthritis.

Sl. No.	Appearance or nature of discharges collected from joints	No. of joints
1	Yellowish, purulent	24
2	Serous fluid with yellowish flocculent material	6
3	Yellow, creamy	4
4	Reddish brown	4
5	Fibrinopurulent	6
6	Yellowish, thick and cheesy consistency	4

3.1.2 Synovial fluid/Joint discharge pH

The mean±SE pH values of synovial fluid/joint discharge samples from calves affected with septic arthritis were 6.13±0.09 and 6.28±0.09 on day 0, and 7.06±0.07 and 7.10±0.06 on day 28 in Group I and Group II, respectively. A statistically significant increase ($P< 0.05$) in pH

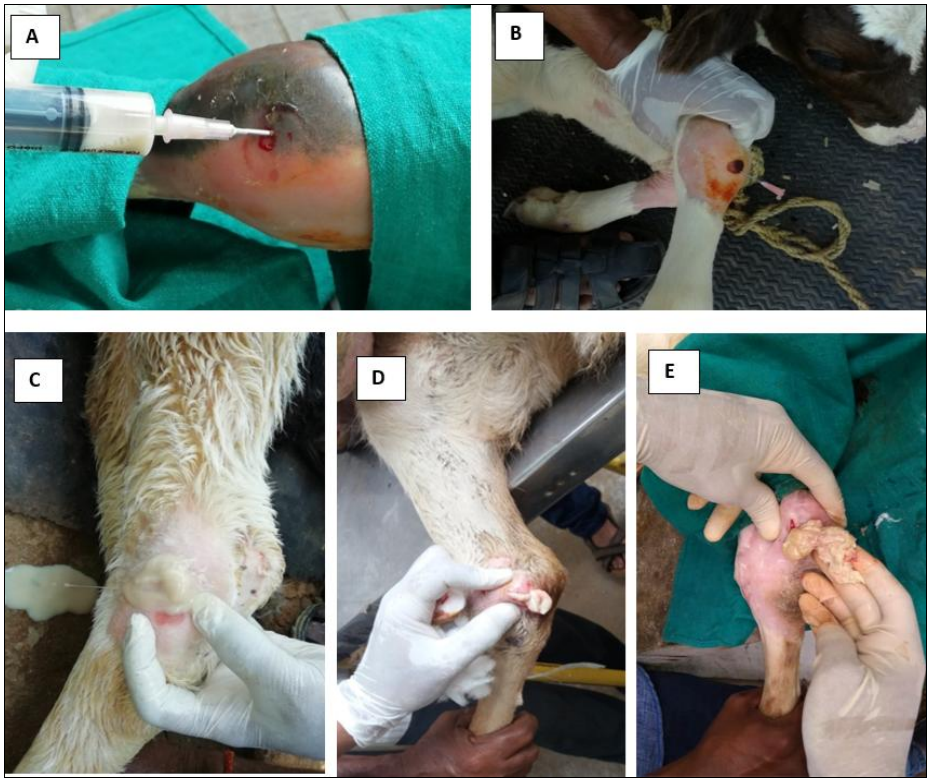

Fig 3: Showing color and consistency of synovial discharge/fluid from with septic arthritis.

(A yellowish, purulent pus; B. light Yellowish with flocculent material; C. Yellowish and creamy consistency pus; D. Yellowish and Thick consistency pus; E. Yellowish and cheesy consistency pus) was observed from day 0 to day 14 in both groups, followed by a non-significant rise thereafter, with values approaching baseline by day 28 (Table 2).

3.1.3 Total protein

The mean±SE concentrations of total protein (g/dl) in synovial fluid/joint discharge samples from calves with septic arthritis were 6.30±0.03 and 5.56±0.18 on day 0, and 1.95±0.08 and 1.76±0.15 on day 28 in Group I and Group II, respectively. Protein concentrations were significantly elevated ($P<0.05$) at the time of presentation compared to the corresponding post-treatment values on day 28 in both groups. A marked reduction in total protein levels was observed between day 0 and day 21 ($P<0.05$), followed by a further non-significant decline, with values approaching baseline by day 28 (Table 2).

3.1.4 Total WBC count

The mean±SE values of total leukocyte count (×10³/µl) in synovial fluid/joint discharge samples from calves with septic arthritis were 33.00±1.18 and 28.83±0.94 on day 0 and 3.67±0.55 and 3.17±0.30 on day 28 in Group I and Group II, respectively. Leukocyte counts were significantly elevated ($P<0.05$) at presentation compared with post-treatment values on day 28 in both groups. A progressive and statistically significant reduction in total leukocyte count was observed from day 0 to day 28 in Group I, and from day 0 to day 21 in Group II, after which values stabilized (Table 2).

3.1.5 Polymorphonuclear cells (PMNs)

The mean±SE percentages of polymorphonuclear cells (PMNs) in synovial fluid/joint discharge samples from calves with septic arthritis were 87.83±1.47 and 92.00±1.34 on day 0, and 10.67±0.49 and 9.83±0.60 on day 28 in Group I and Group II, respectively. A statistically significant reduction in PMN percentage was observed from day 0 to day 28 in Group I and up to day 21 in Group II ($P<0.05$). By day 28, PMN levels had declined to baseline values consistent with the normal physiological range (Table 2).

Table 2: pH, Total protein, total leucocyte count, polymorphonuclear cells recorded at different time intervals in group I and group II animals (Mean±S.E.)

Sl. No	parameters	Days	0	7	14	21	28
1	pH	Group I	6.13±0.09^{a}	6.40±0.10^{a}	6.78±0.09^{b}	6.93±0.05^{b}	7.06±0.07^{b}
		Group II	6.28±0.09^{a}	6.43±0.06^{a}	6.88±0.11^{b}	6.98±0.07^{b}	7.10±0.06^{b}
2	Total protein (g/dl)	Group I	6.30±0.03^{a}	3.83±0.12^{a}	2.86±0.11^{c}	2.12±0.10^{d}	1.95±0.08^{d}
		Group II	5.56±0.18^{a}	2.76±0.08^{b}	2.39±0.15^{bc}	1.97±0.09^{cd}	1.76±0.15^{d}
3	Total WBC count (1000/mm^3)	Group I	33.00±1.18^{a}	20.67±0.98^{b}	12.83±1.01^{c}	7.67±0.42^{d}	3.67±0.55^{e}
		Group II	28.83±0.94^{a}	21.33±1.05^{b}	12.50±0.16^{c}	5.83±0.60^{d}	3.17±3.30^{d}
4	PMNs (%)	Group I	87.83±1.47^{a}	57.33±2.02^{b}	34.83±1.77^{c}	21.33±1.92^{d}	10.67±0.49^{e}
		Group II	92.00±1.34^{a}	37.33±1.89^{b}	21.83±1.35^{c}	16.33±1.43^{d}	9.83±0.60^{d}

Means bearing different superscripts differ significantly (p<0.05)

Discussion

In the present study, the color of synovial fluid/joint discharge at presentation ranged from colorless to pale yellow, thick yellowish, or reddish-brown. Physiologically, normal synovial fluid is clear, colorless to straw-colored, and non-coagulable due to the absence of fibrinogen and plasma clotting factors (Krishnamurthy and Tyagi, 1970; Greenough and Weaver, 1981; Weaver, 1997) [15-17]. In contrast, infected synovial effusions have been described as turbid, amber to hemorrhagic, and containing fibrinous flocculent material (Van Pelt, 1970 [18]). Consistent with these reports, the majority of samples in this study were turbid, purulent, and contained fibrinous flocculations, likely attributable to cellular accumulation, fibrin, degenerated cartilage fibrils, and debris (Tulamo *et al.*, 1989; Perman, 1980; Weaver, 1997) [19, 20, 17]. By day 14 post-treatment, synovial fluid in both groups had become clear and viscous, indicating effective resolution of infection-associated exudates, fibrin and cellular debris.

In the present study, synovial fluid pH decreased in association with joint infection, consistent with previous findings in humans and dogs, where infectious arthritis is characterized by markedly reduced pH values (Sawyer, 1963; Ward and Steigbigel, 1978; Tulamo *et al.*, 1989) [21, 22, 19]. Initially, the synovial fluid was acidic; however, values gradually shifted toward a slightly alkaline range following treatment, indicating resolution of infection and restoration of joint homeostasis.

In the present study, the total protein concentration of synovial fluid/joint discharge was significantly elevated at presentation in both groups compared to their respective baseline values on day 28. A progressive decline was observed following initiation of treatment, with values returning to baseline by day 21. Similar increase in synovial protein content have been reported in cattle with experimentally induced septic arthritis (Kumar and Singh, 1995; Chawla *et al.*, 1989) [13, 23] and in clinical cases (Van Pelt, 1970; Ndikuwera *et al.*, 1989; Rohde *et al.*, 2000) [18, 24, 25]. The present findings are consistent with those of Rohde *et al.* (2000) [25], Desrochers (2004) [7], Lugo and Gaughan (2006) [26], Ramanathan (2007) [27] and Altintas *et al.* (2010) [14]. In this study, synovial protein levels at presentation were nearly three times higher than the corresponding values on day 28, reflecting the severity of the inflammatory process as described by Van Pelt (1970) [18] and Anderson and Liberg (1980) [28]. Such elevations are attributed to alterations in synovial membrane permeability and disruption of the synovial-blood barrier, facilitating the influx of plasma proteins, particularly globulins, into the joint cavity (Bertone, 1996) [29].

In the present study, synovial fluid total WBC count at presentation was significantly elevated in all joints of both groups compared to baseline values on day 28 of postoperative treatment (33.00 and 28.83 $\times 10^3/\mu l$ in Group I and 338.17 $\times 10^3/\mu l$ in Group II, respectively). These findings are consistent with earlier reports (Orsini, 1984 [30]; Ndikuwera *et al.*, 1989; Howard, 1993; Pal *et al.*, 1994; Kumar and Singh, 1995; Butt, 2000; Rohde *et al.*, 2000) [30, 24, 31, 32, 13, 33, 25]. Cytological examination of synovial fluid is regarded as the most reliable diagnostic indicator of joint infection (Rohde *et al.*, 2000) [25], with total nucleated cell counts exceeding 30,000 cells/µl considered pathognomonic for septic arthritis (St-Jean, 1993; Rohde *et al.*, 2000) [12, 25]. Leukocytosis reflects the extent of inflammation within the synovium (Tulamo *et al.*, 1989) [19], largely due to disruption of the blood-synovial barrier and influx of plasma proteins and inflammatory mediators such as kinins, histamine, complement and fibrinolytic enzymes (plasminogen, trypsin). Following treatment, WBC counts progressively declined to baseline levels by day 28 in both groups, demonstrating the efficacy of chitosan-ceftiofur sodium and honey in resolving infection and eliminating inflammatory debris and fibrin deposits.

In the present study, synovial fluid polymorphonuclear (PMN) cell counts were significantly elevated at presentation in both groups (87.83 and 92.0 in Group I and II, respectively) compared to baseline values on day 28, consistent with findings of Ramanathan (2007) [28]. The early influx of PMNs represents the host's initial response to eliminate foreign organisms, typically occurring within 24 hours of contamination. The observed increase in PMN proportion is likely attributable to vasodilation in the synovial subintima and chemotactic-mediated migration of neutrophils into the joint cavity. Following treatment, PMN counts gradually declined, reflecting clearance of inflammatory exudates and the effect of parenteral antibiotics (Bertone, 1996) [29].

Conclusion

The present study demonstrates that septic arthritis in calves induces marked alterations in synovial fluid characteristics, including turbidity, acidic pH, elevated total protein, and increased total WBC and PMN counts. These parameters serve as reliable indicators of the severity of joint inflammation and infection. Monitoring changes in synovial fluid pH, total protein, total leukocyte count and PMN proportion provides valuable diagnostic and prognostic information, supporting early detection and effective therapeutic management of septic arthritis in calves.

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Author's Contribution

Not available

Conflict of Interest

Not available

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