Original Article
Clinicopathological Evaluation of Naturally Occurring Septic Arthritis in the Bovine Calves

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ABSTRACT

Background: Septic arthritis affects ruminant welfare because, if left untreated, it can cause chronic pain and limit the mobility of affected joints.

Objectives: This study aimed to investigate the clinical and pathological changes in arthritic bovine calves.

Methods: The study was conducted on 12 calves with swollen knees or carpal joints. All calves were evaluated through clinical, radiographic, and ultrasonographic examination. Peripheral blood was aspirated from each to assess hematobiochemical changes. Synovial fluid and infected swab samples were subjected to bacteriological analysis, and a synovial biopsy was taken for histological examination.

Results: Ultrasound revealed inflammatory effusions with various echogenicity in the afflicted joint capsule, while radiography showed remarkable swelling of joints and surrounding structures and the development of new bone. Regarding hematological variables, the value of total erythrocyte count, total leukocyte count, and erythrocyte sedimentation rate significantly (P<0.05) increased in septic arthritic calves compared to healthy calves. In the arthritis group, the serum concentration of alanine transaminase, alkaline phosphatase, and aspartate aminotransferase was considerably (P<0.05) higher than in healthy calves. The total protein and urea values were significantly (P<0.05) decreased in calves with infected arthritis. From the synovial fluid and purulent discharge of the joints, *Staphylococcus aureus* and *Escherichia coli* were isolated. Histopathology of synovial tissue revealed chronic suppurative inflammation with intense hyperplasia of joint synovium.

Conclusion: The results of this study may aid veterinarians in effectively diagnosing and treating septic arthritis in calves.

Keywords: Calves, Histopathology, Radiography, Septic arthritis, Ultrasonography

How to Site This Article

1. Introduction

Septic arthritis is characterized as joint inflammation brought on by invading microbial pathogens into the joint area (Desrochers et al., 2001). Inflammation is categorized as acute or chronic based on its clinical history and characteristics (Gokhan & Ozturk, 2016). Small and large ruminants are most frequently affected by arthritis (Desrochers & Francoz, 2014). Depending on the severity of the disease, arthritis can affect a single joint, multiple joints, or even the entire body (Temizsoylu & Yigitarslan, 2015). Different degrees of distortion in the joint’s parts occur in septic arthritis, leading to functional issues, including lameness, trouble standing, swelling, and discomfort (Haerdi-Landerer et al., 2010). Synovial infection and severe joint pain can disrupt the natural functioning of a joint, leading to the premature and irreversible breakdown of cartilage and bone, thus preventing the joint from fully recovering (Mulon et al., 2016; Adams et al., 2014).

Lameness caused by septic arthritis is a common, expensive, and widespread health problem in cattle. Septic arthritis impacts ruminant welfare because, if not treated effectively, it can cause severe discomfort that lasts long and impairs joint function and range of motion (Jesse et al., 2017). Early diagnosis and appropriate disease management are crucial for reducing the probability of culling the afflicted animals and restoring the joints’ normal physiology (Adams et al., 2014). Clinical features, biochemical and microbiological blood and synovial fluid examination, and diagnostic imaging are frequently employed to identify the illness (Desrochers & Francoz, 2014). Ultrasonography and radiography are important for evaluating joint disorders because they reveal details on the joint’s location, size, and structure (Desrochers & Francoz, 2014). Therefore, the present research has been conducted to assess radiographic and ultrasonographic findings of infected joints in calves, along with hematobiochemical, bacteriological, and histopathological changes of the infected synovium during the active cascade of septic arthritis.

2. Materials and Methods

Experimental animals

The study was conducted on two groups of bovine calves: Arthritic and healthy control. The animals of this clinical study included 12 calves of different breeds, ages, and sex which were brought to the Veterinary Teaching Hospital of Bangladesh Agricultural University, from January to December 2020 with swelling and pain in carpal or knee joints, with gait difficulties.
Histopathological examination

Synovial tissue biopsies were collected from the affected joint. The specimens were fixed in 10% formalin for 48 h, and after a series of alcohol and xylene treatments, the specimens were embedded in paraffin. Five-micrometer thick sections were cut by microtome (Histoline®, USA) and stained with hematoxylin and eosin. The slides were then examined using a photographic microscope (Micros®, Austria).

Statistical analysis

Statistical analysis was performed using the independent samples t-test in SPSS software, version 20. Data were expressed as Mean±SE. A P≤0.05 was considered statistically significant.

Table 1. Hematological findings of calves associated with infected arthritis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Control</th>
<th>Septic Arthritis Calves</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC (10^6/mm³)</td>
<td>5.8±0.057</td>
<td>7.62±0.006</td>
<td>0.000</td>
</tr>
<tr>
<td>TLC (10^9/mm³)</td>
<td>9.1±0.057</td>
<td>9.73±0.046</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>9±0.11</td>
<td>7.86±0.067</td>
<td>0.001</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33±0.57</td>
<td>34±0.577</td>
<td>0.288</td>
</tr>
<tr>
<td>ESR% (1st hour)</td>
<td>2.1±0.057</td>
<td>1.1±0.057</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Abbreviations: TEC: Total erythrocyte count; TLC: Total leukocyte count; Hb: Hemoglobin; PCV: Packed cell volume; ESR: Erythrocyte sedimentation rate.

3. Results

Radiographic and ultrasonographic findings

Radiographic examination demonstrated remarkable swelling of the synovial membrane and associated structures leading to the narrowing of joint space (Figure 1a). There were increased opacity and joint compression due to increased purulence, intra-articular constriction, and degeneration on joint surfaces (Figure 1b).

When afflicted joints were seen with ultrasound, they often exhibited larger joint spaces and corpuscular reflecting bodies with strong hyperechogenicity in the synovial fluid. The synovial fluid in these individuals was diverse and seemed hyperechogenic (Figure 2a). Some calves with infected arthritis showed smooth articular cartilage surfaces with highly noticeable hyperechoic lines (Figure 2b).

Table 2. Clinical chemistry of healthy and arthritis calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Control</th>
<th>Septic Arthritis Calves</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.91±0.01</td>
<td>4.02±0.015</td>
<td>0.004</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.92±0.02</td>
<td>5.23±0.03</td>
<td>0.000</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>75.30±0.65</td>
<td>973.77±1.92</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>6.66±0.24</td>
<td>10.11±0.56</td>
<td>0.005</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>12.24±0.62</td>
<td>54.45±1.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.09±0.04</td>
<td>0.97±0.01</td>
<td>0.048</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>61.16±0.72</td>
<td>10.70±0.21</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransferase.
Changes in blood profile in calves with septic arthritis

The changes in the routine blood profile in the calves with septic arthritis are presented in Table 1. In this study, calves with septic arthritis exhibited varying degrees of alteration in the mean values of several hematological parameters. In comparison to healthy controls (5.8±0.057), the levels of TEC were significantly (P<0.05) elevated in septic arthritis calves (7.62±0.006). The values of TLC were significantly (P<0.05) higher in septic arthritis calves (9.73±0.046) compared with healthy calves (9.1±0.057). On the other hand, in septic arthritis calves,

Figure 1. Radiograph of septic joint

a) Increased in the volume of the joint due to extensive swelling of synovium and associated structure of affected joints along with subchondral bone necrosis, b) Widening of the lateral part of fetlock joint and luxation of the first phalanx was evident

Figure 2. Ultrasound scanning result of infected arthritis joint

a) The synovial space seems hyperechogenic and heterogenic, b) The synovial space and corpuscular patches in the synovial fluid look complex echogenicity and heterogenic
the Hb (7.86±0.067) and ESR (1.1±0.057) levels were substantially lowered (P<0.05) compared to those of healthy animals. The PCV level showed no discernible difference between the two groups of calves.

Clinical chemistry in calves associated with septic arthritis

The alterations in clinical chemistry in normal and arthritis calves are shown in Table 2. We observed that the mean values of the biochemical profile in the two groups were remarkably different. As compared with healthy calves, septic arthritis calves had (P<0.05) higher recorded Mean±SE values of serum albumin (3.91±0.01 to 4.02±0.015), ALT (6.66±0.24 to 10.11±0.56), AST (12.24±0.62 to 54.45±1.28), and ALP (75.30±0.65 to 973.77±1.92). On the other hand, in septic arthritis calves, the recorded value of serum total protein (5.23±0.03), creatinine (0.97±0.01), and urea (10.70±0.21) were significantly lower than those in healthy calves.

Figure 3. Morphological characteristics of isolated bacteria

a) Rod-shaped clustered in a single or short chain-like fashion indicated the colony of *E. coli*, b) Gram-positive, spherical shaped in cluster form indicated the colony of *S. aureus*

Figure 4. Histopathological findings demonstrating chronic suppurative inflammation, with profound hyperplasia of the joint synovium and extensive fibrosis

Severely inflamed periarticular tissues were also noted, and these were demarcated by caseous necrosis area (H&E stain, original magnification 100× (a) and 400× (b, c).
Bacteriiological evaluation

Microbiological examination revealed *Escherichia coli* and *Staphylococcus aureus* in the synovial fluid and swab samples taken from the calves with septic arthritis. On mannitol salt agar, the *S. aureus* produced a golden yellow colony, while on MacConkey Agar, the *E. coli* had a deep pink colony indicating the presence of these pathogens. On gram staining, *E. coli* manifested rod-shaped clusters in a single or short chain-like fashion (Figure 3a). However, *S. aureus* exhibited gram-positive spherical shapes in clusters (Figure 3b).

Histopathological findings

Histopathological evaluation revealed severe, chronic, multifocal cellulitis (Figure 4). There was chronic perivascular inflammation and severe degenerative changes, including fibrosis. There was marked synovial hyperplasia, and the synovial stroma exhibited inflammatory cells, granulomatous tissue, and fibroblasts. The inflammation extended to the subchondral bone.

4. Discussion

Septic arthritis frequently results from systemic infections such as umbilical diseases or direct trauma. Many animals are slain each year because of the prolonged treatment duration and high expenditures. Clinical symptoms, radiography, ultrasonography, and bacterial cultures are used to diagnose septic arthritis. Radiographs may not be effective in acute instances because bony changes might not be noticeable for 5-7 days; however, they are beneficial in chronic situations because they show the severity of the osteomyelitis and help with debridement (Gokhan & Ozturk, 2016). In this study, radiographic examination revealed swelling of joint tissues, including synovium leading to narrowing of joint space. Constant et al. (2018) and Nuss, (2011) found similar changes in the radiography of infected joints due to fluid and gas accumulation. Temizsoylu and Yigitarslan (2015) reported subchondral bone lysis, periostitis, osteomyelitis, and osteophytic formations in joint space in the case of chronic patients. Similar findings were observed for chronic cases in the present study.

Early diagnosis of arthritis is challenging before clinical symptoms appear. However, diagnostic ultrasonography can identify arthritis without clinical symptoms (Gokhan & Ozturk, 2016). In acute septic arthritis, the synovial fluid increases volume, and echogenic (gray) material (fibrin) can float in the joint. Cartilage is anechogenic (black) because of its high content of water, but the subchondral bone is hyperechogenic (white), and lysis or defect will change its contour (Paakkonen & Peltola, 2013). Ultrasonographic examination of this study exhibited enlarged joint and synovial effusion. This study also revealed the hyperechogenic and heterogeneous appearance of synovial fluid due to corpuscular reflective bodies in the synovium. According to previous reports, ultrasonographic examination revealed various changes in synovial fluid volume, the echogenic appearance of synovial fluid, synovial membrane, joint surface, and joint relationship of joint with peripheral tissue. Gorgul et al. (2010) and Desrochers and Francoz, (2014) found increased synovial fluid volume and echogenic appearance of synovial fluid. Yurdakul (2019) and Tak et al. (1997) observed that the synovial fluid had a hyperechogenic appearance and heterogeneous structure, with corpuscular reflective bodies showing intense hyperechogenicity in the synovial fluid in 18 cases and that the articular cartilage surface formed a hyperechoic line and had a smooth surface in 6 cases.

Regarding hematological changes, in this study, TEC, TLC, and ESR increased in septic arthritis calves compared to their healthy counterparts. Kumar et al. (2018) reported a significant increase in TLC and ESR values during infectious joint illness which agreed with this finding. The significant increase in TLC and ESR might be due to increased inflammatory cellular infiltration and bacterial infection. In this study, the level of Hb was significantly decreased in septic arthritis calves than in healthy calves. These findings revealed that the affected calves had become severely dehydrated. In this study, the result of liver enzymes, including ALT, AST and ALP, and albumin, showed a significant increase in septic arthritis calves when compared to healthy calves, which might be related to possible hepatic dysfunction persuaded by the inflammatory response (Bumin et al., 2001; Kumar et al., 2018; Yurdakul, 2019). Serum creatinine and urea level were significantly decreased in affected calves. The serum concentration of total protein level was significantly reduced. According to Tamura et al. (2016), this decrease might be associated with concomitant hypoglobulinemia caused by an inflammatory illness (septic arthritis) that resulted in impaired protein metabolism and abnormalities in liver functions and or abnormalities in metabolism and anorexic state of afflicted animals (Tak et al., 1996; Turunen et al., 2016). This condition stimulated the synthesis of different globulin fractions (Haerdi-Landerer et al., 2010).

It is uncommon to do or recommend bacterial culture. In cattle, only 60% of septic arthritis cases are culture-positive; however, the success rate in isolation increases...
with fluid volume cultured. The most frequent isolates are Arcanobacterium pyogenes in older calves, chronic infections, and *E. coli* in newborns and acute cases (Carpenter et al., 2011). It has been estimated that 50% of total arthritis is caused by bacteria such as *S. aureus*, *Streptococcus pneumoniae*, *E. coli*, *Proteus* sp., *Salmonella* sp., *Serratia marcescens*, *Erysipelothrix rhusiopathiae*, *Chlamydia* sp. and *Neisseria* sp. (Colavite & Sartori, 2014). Bacterial arthritis occurs most commonly in food animals (Desrocher & Francoz, 2014), especially in young animals. In the present study, microbiological examination of swab and synovial fluid samples revealed *S. aureus* and *E. coli* as the causative agents of septic arthritis, which is in agreement with previous reports (Colavite & Sartori, 2014; Desrocher & Francoz, 2014).

Histopathological findings demonstrated chronic suppurative inflammation with profound hyperplasia of the joint synovium and extensive fibrosis. Severely inflamed periarticular tissues were also noted. These findings are supported by previous reports (Nikkari et al., 1995; Zvaifler & Firestein, 1994), where they reported inflammation and fibrosis in periarticular tissue.

The study had limitations, such as the inability to conduct a hematobiochemical analysis to explain the variations by considering the breed, age, and sex of the animals.

5. Conclusions

Based on the findings of this study, radiographic and ultrasonography examinations and bacteriological and histological examinations of infected joints are useful tools for diagnosing septic arthritis in calves. Nevertheless, early detection and treatment are necessary to improve the outcome of septic arthritis in calves.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were performed following the standards outlined in the guidelines of the Animal Welfare, Ethics and Experimentation Committee (AWEEC, permission number: AWEEC/BAU/202 2[34]) of the Faculty of Veterinary Science, Bangladesh Agricultural University.

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Authors' contributions

Study design, supervision and editing the manuscript: Mahmudul Alam. Data collection: Antora Akter, Sabuj Rahman and Abu Hanif; Data acquisition and writing manuscript: Antora Akter and Sabuj Rahman; Editing manuscript and performing microbiological work: Marzia Rahman; Conducting ultrasonographic examination and interpreting the images: Nasrin Sultana Juwena.

Conflict of interest

The authors declared no conflict of interest.

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