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## **CLINICOPATHOLOGIC FINDINGS AND TREATMENT OF BOVINE ACTINOMYCOSIS**

(With One Table and 5 Figures)

By

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### **SUMMARY**

The objective of the current study was to characterize the clinical and pathological findings of actinomycosis in cattle, and to assess their response for treatment. Actinomycosis was clinically diagnosed in nine cattle. The lesions appeared as hard immobile swellings in the mandible and rarely in maxilla. Smears made from crushed granules in pus revealed the presence of sulfur granules that appeared microscopically as club-like rosettes. Hematological and biochemical examinations indicated leukocytosis, neutrophilia, hyperprotenemia, hypoalbuminaemia and hyper gammaglobulinemia. Treatment through parenteral administration of streptomycin at a dose rate of 15mg/kg daily given intramuscularly for 2 weeks was carried out. Surgical intervention through pus aspiration with long sharp needle and washing with normal saline followed by flushing with iodine solution 2%, was also tried. Adjunct therapy of broad-spectrum antibiotic and non-steroidal anti-inflammatory was administered intramuscularly for five days as a post-operative measure. Four out of nine treated cattle (44.4%) were responded for treatment and almost recovered completely within four months.

***Keywords:*** *actinomycosis, biochemical analysis, cattle, clinicopathological findings, hematological examinations, treatment.*

## INTRODUCTION

Actinomycosis (lumpy jaw) in cattle is a chronic infectious disease characterized by suppurative granulation of the skull, particularly the mandible and maxilla. There are gross painful swellings; abscesses, fistulous tracts and extensive fibrosis that all contribute to the granulomatous lesion (Radostits *et al.*, 2007).

The classical lesions of lumpy jaw were described for the first time by Bollinger (1877). The causative organism of lumpy jaw is *Actinomyces bovis*, a Gram-positive filamentous anaerobe. It is a normal inhabitant of the oral flora and upper respiratory and digestive tracts of most animals (Smith, 2002). The organism gains access to the soft tissues as a result of mucosal damage caused by sharp objects or erupting teeth. Following the infection, a proliferation of connective tissue, invasion with leucocytes and the resulting formation of a walled tumor-like mass can be seen. The granuloma then invades the bones of the mandible or occasionally the maxilla (Eddy, 2004).

Actinomycosis is characterized by the presence of ‘sulphur granules’, which contain bacteria that are arranged in clubs or phagocytized (Pine & Overman 1963). The pathogenesis of the disease in cattle is traumatic and the route of entry is typically a break of the mucosa of the gingiva due to penetrating small and hard vegetable bodies. The infection causes periostitis and, in most cases, osteomyelitis arising from periodontitis (Palmer 1993).

Fine-needle aspiration biopsy (FNAB) is a less aggressive procedure than histological biopsy and might be of aid in recognizing microorganisms and sulphur granules, especially when bacteriological analyses are negative. FNAB is a safe, easy-to-use, fast, effective, inexpensive and minimally invasive diagnostic technique that can be performed before microbiological examination and anti-microbial therapy. FNAB might be useful for the differentiation of actinomycosis lesions and neoplastic disorders of bone (Militerno, 2008).

The current study describes the clinicopathological findings of actinomycosis in cattle and emphasizes the usefulness of pus aspiration with long sharp needle and washing with normal saline followed by flushing with iodine solution 2%.

## **MATERIALS and METHODS**

### **Animals, history and physical examination**

Nine cattle were examined in this study. They included five cows, one bull (aged 5-9 years old, weighed 370-680 kg) and three calves (aged 1-5 months, weighed 45-80 kg). The three calves and four cows were examined at The Veterinary Teaching Hospital, Rakuno Gakuen University, Japan. The remaining two cows were examined at The Veterinary Teaching Hospital, Qassim University, Saudi Arabia. Animals were referred to the hospitals because of inappetance, loss of body condition, swelling of maxilla or mandible. According to the owners, duration of illness ranged from 1 to 10 weeks. All animals underwent a thorough physical examination (Radostits *et al.*, 2000), which included general behavior and condition, auscultation of the heart, lungs, rumen and intestine, counting of heart and, respiratory rates and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination.

### **Hematological examinations and biochemical analyses**

Two blood samples were collected by puncturing the jugular vein, one on EDTA and the other without anticoagulant. The first blood sample was used to analyze the haematological parameters including haematocrit, hemoglobin, total and differential leukocyte. The second blood sample was centrifuged (3000 rpm for 10 minutes) and serum samples were collected and frozen at  $-20^{\circ}$  C. The electrophoresis was used to determine serum protein fractions. Reference values were according to (Kaneko *et al.*, 1997; Radostits *et al.*, 2007).

### **Treatments**

Treatment consisted of injection of streptomycin at a dose rate of 15mg/kg daily given intramuscularly for 2 weeks. Lesions were aspirated with a sharp, long needle and the pus was removed under deep sedation with xylazine 2% (0.1 mg/kg IV, Rompun, Bayer HealthCare, Germany) and local anesthesia with 2% lignocaine hydrochloride (Xylocaine polyamp 2%, AstraZeneca, Japan). Lesion cavity was then washed 3 times with normal saline and finally flushed twice with iodine solution 2%

(Figure 3). This procedure was repeated weekly for 4 weeks. After procedure, 5-day care included parenteral administration of antibiotic (10mg/kg IM, Oxytetracyclin, Terramycin LA<sup>®</sup>, Pfizer), non-steroidal anti-inflammatory drug (2.2 mg/kg IV, flunixin meglumine, Finadyne<sup>®</sup>, Schering-Plough Animal Health) and Glucose 10%.

### **Necropsy**

One calf and 4 cows did not respond to treatment and therefore were necropsied.

### **Statistical analysis**

Data are presented as mean  $\pm$  SD and the analysis was conducted using SPSS program, version 16.0 (2007). Hematological and biochemical data were compared using Student *t* test. The Level of significance was set at  $P < 0.05$ .

## **RESULTS**

### **Clinicopathological findings**

Actinomycosis lesion appeared as hard and immobile swellings in the mandible in 2 calves and 6 cows; in one calf only in the maxilla. Except in one cow, lesions were painless. In two cows, sticky, honey-like fluid containing minute, hard, yellow-white granules were detected. In one cow, a severe form was observed where lesions involved the muscles and fascia (Figure 1). The general condition of two cows was poor. In cows, milk yield was severely decreased to a few liters per day. Values of rectal temperature, pulse and respiratory rates were  $38.9 \pm 0.7^{\circ}\text{C}$ ,  $85 \pm 22/\text{m}$  and  $25 \pm 10/\text{m}$ , respectively.

Smears made from crushed granules in pus revealed the presence of sulfur granules that appeared microscopically as club-like rosettes (Figure 2).

### **Hematological examinations and biochemical analyses**

Complete blood count findings included a neutrophilic leukocytosis. Abnormalities identified from the chemistry profile included

hyperprotenemia, hypoalbuminemia and hyper gammaglobulinemia (Table 1).

## **Treatment**

Four out of nine treated cattle (44.4%), two calves and two cows, were responded for treatment and almost recovered completely within four months (Figure 4).

## **PM findings**

Post-mortem examinations revealed bones rarefaction with the presence of a sinuses containing thin, whey-like pus with small, gritty granules. Other necropsy lesions included hydrothorax, abomasal and intestinal edema, and abdominal lipomatosis (Figure 5).

**Table 1:** Hematological and biochemical findings in cows with actinomycosis

Parameters	Finding ( $n = 9$ )	Reference value
Hematocrit (%)	29±7	24-46
Hemoglobin (g/dL)	10.5±1.7	8.0-15.0
Leukocyte count (/□ L)	17500±7300*	4000-12000
Neutrophils (/□ L)	12400±1250*	600-4000
Lymphocytes (/□ L)	3900±17	2500-7500
Total protein (g/dL)	8.9±1.5*	6.7-7.5
Albumin (g/dL)	2.8±0.6	5.7-8.1
α-globulin (g/dL)	1.1±0.2	2.1-3.6
β-globulin (g/dL)	1.3±0.2	0.75-0.88
□ □ globulin (g/dL)	3.7±0.5**	0.8-1.1

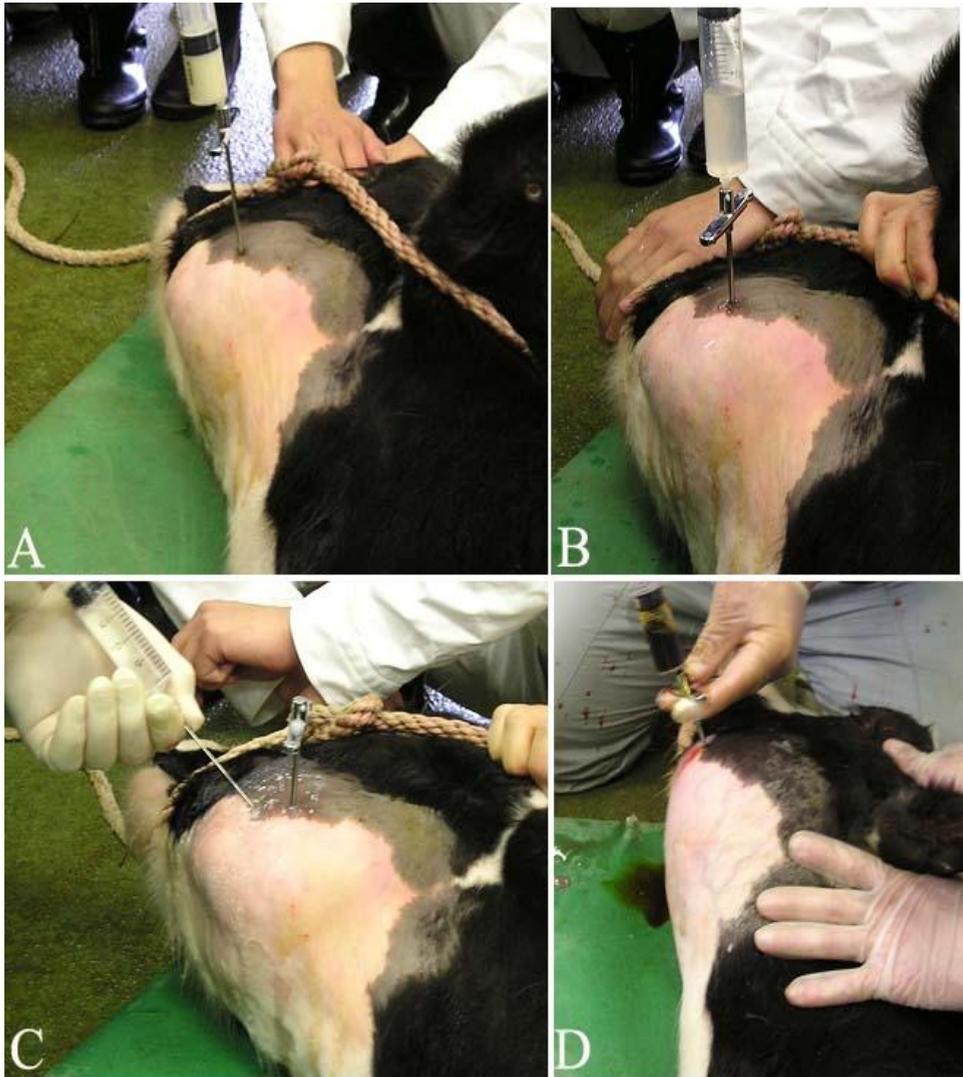
\*P < 0.05; \*\*P < 0.01.



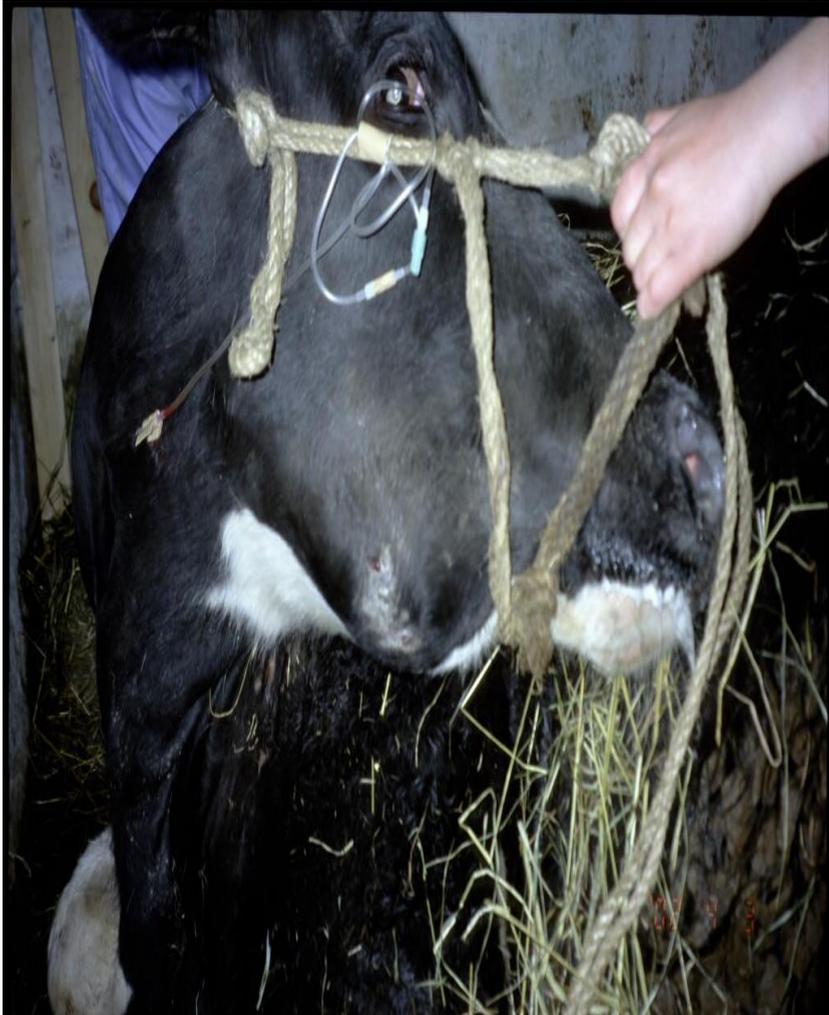
**Fig. 1:** Clinical presentations in a calf (A) and 3 cows (B, C, D) with actinomycosis. In the calf, the lesion was located bilaterally in the maxilla. Actinomycosis lesion in the cows appeared as hard and immobile swellings in the mandibles. In one cow (D), a severe form was observed where lesions involved the muscles and fascia.



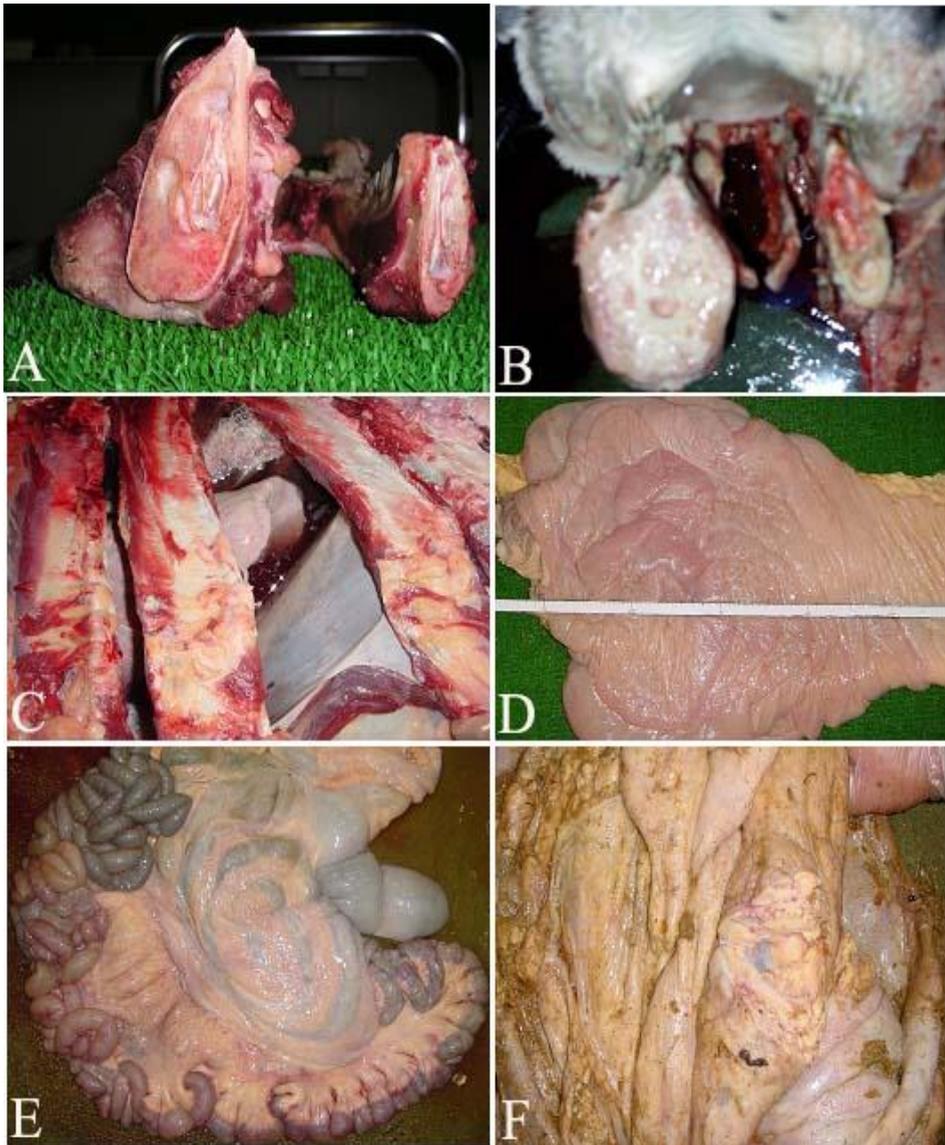
**Fig. 2:** Actinomycosis in a 5-month-old calf. Smears made from crushed granules in pus revealed the presence of sulfur granules that appeared microscopically as club-like rosettes.



**Fig. 3:** Aspiration of actinomycosis lesions in the mandible of a 5 month old calf. Lesions were aspirated with a sharp, long needle and the pus was removed (A). Lesion cavity was then washed 3 times with normal saline (B&C) and finally flushed with iodine solution 2% (D).



**Fig. 4:** Post-treatment in a cow with actinomycosis. Animal is almost completely recovered after 4 months.



**Fig. 5:** Necropsy findings in cattle with actinomycosis. Rarefaction of the bones (A) with presence of sinuses containing thin, whey-like pus with small, gritty granules (B) were detected. Other necropsy lesions included hydrothorax (C), abomasal (D) and intestinal (E) edema and abdominal lipomatosis (F).

## DISCUSSION

Bovine actinomycosis, commonly known as "lumpy jaw", usually involves the bones of the head, particularly the lower jaw. Actinomycotic infections of the softer tissues occur in cattle but are rare. Large, pus-filled tracts or cavities (abscesses) form when the infection spreads through the bone. Connective and other body reparative tissues in and near the bone grow abundantly in an attempt to wall off the abscess. By the time the infection has extended from the bone to the soft tissue and skin to establish a fistula or drain for the lesion. The involved parts of the jaws may be enlarged 2 or 3 times. Fistulas from abscesses of the bones of the head sometimes extend inward and discharge into the pharynx or mouth. The palate and gums next to the bones often are swollen and inflamed. The teeth may loosen (Bertone & Rebhun, 1984; Raymond & Foglia, 1998; Radostits *et al.*, 2007; Van Metre *et al.*, 2007). In the current study, actinomycosis lesions appeared as hard and immobile swellings in the mandible in two calves and six cows; in one calf only in the maxilla. Except in one cow, lesions were painless. A severe form of the disease was observed in one cow, where lesions involved the muscles and fascia. Lacerations of the gums, and the trapping and decay of foodstuffs in the oral cavity accompany the eruption of the teeth during may be the explanation of the occurrence of the disease in three young calves. Injuries of the oral mucosa by a dry, harsh, rough feeds may act as a predisposing factor for the occurrence of the disease in six adult cattle.

Farooq *et al.* (2010) described a typical case of bovine actinomycosis in a seven-month pregnant heifer with a hard swelling on the middle of the maxilla bone at the level of the central molar teeth. Tentative diagnosis was made through clinical signs. In this study, diagnosis of the disease depended on the presence of a small, hard, yellowish granules "sulfur granules" or "rosettes," which observed as tiny grains in the pus from abscesses of all examined cases. After crushing of the washed granules on a glass slide and staining with gram stain, Gram-positive micro-organisms were seen either as short rods, filaments, or branching forms (club-like rosettes). Neutrophilic leukocytosis detected during hematological examinations almost due to the bacterial infection. Results of biochemical analysis (hyperprotenemia and hypergammaglobulinemia) may indicate chronic inflammatory process.

Rarefying osteitis, osteoporosis interspersed with granulomatous tissue and pockets of thin pus containing, yellow, sand-like granules are the main pathological changes found in bovine actinomycosis. Diagnosis is

based on the clinical signs. Absolute diagnosis requires, however a tissue core biopsy or fluid aspirate to identify the causative organism by staining the crushed yellow granules found in the pus and demonstrating the Gram-positive filamentous rods. Radiographs confirm osteomyelitis with multiple radiolucent zones and proliferation of periosteal bone (Van Metre *et al.*, 2007). In the present study the main PM findings were bones rarefaction with the presence of a sinuses containing thin, whey-like pus with small, gritty granules. Hydrothorax, abomasal and intestinal edema, and abdominal lipomatosis were also observed.

Treatment of bovine actinomycosis includes oral or intravenous dosing of iodides and/or antibiotics such as penicillin and streptomycin but with variable results (Brunton *et al.*, 2005; Radostits *et al.*, 2007). Since the iodide dosing is time consuming and the antibiotics have poor penetration into the site of the infection (Radostits *et al.*, 2007), effective treatment of lumpy jaw is still awaited. Leaper (2000) and Mettler *et al.* (2009) suggested that treatment of any sort is more likely to be of value when combined with surgical intervention. In the present study surgical intervention, through the usefulness of pus aspiration with long sharp needle and washing with normal saline followed by flushing with iodine solution 2% and administration of broad-spectrum antibiotic and non-steroidal anti-inflammatory intramuscularly for five days as a post-operative measure were an effective method for proper treatment of bovine actinomycosis. For the treatment of actinomycosis in a seven-month pregnant heifer, the area was incised under local anesthesia and debridement of the wound was achieved by sharp surgical debridement and mechanical debridement. Pus, having the appearance of sulphur granules, was completely removed from the excised cavity, which was closed by applying mattress sutures. Adjunct therapy of broad-spectrum antibiotic was administered intramuscularly for five days as a post-operative measure. Catamnesis revealed that the healing was complete in 15 days with no recurrence and untoward consequences (Farooq *et al.*, 2010).

Actinomycosis, or lumpy jaw, is a significant cause of economic losses in livestock because of debilitation of the affected cattle, widespread occurrence of the disease and poor response to the routine clinical treatment. Abrasions or lacerations in the oral cavity, caused by dry, harsh, rough feeds, are believed to be the primary portal of entry for infections of actinomycosis. To prevent the occurrence of this disease should avoid dry, harsh, rough food stuffs which may cause stab of the oral mucosa. Also, affected animals of all species should not be permitted to remain in pastures or feed lots with healthy cattle. This will prevent pus from open

lesions from contaminating food, water, bedding, or cuts and abrasions in non-infected animals.

In conclusions, surgical intervention, through the usefulness of pus aspiration with long sharp needle and washing with normal saline followed by flushing with iodine solution 2% and administration of broad-spectrum antibiotic and non-steroidal anti-inflammatory intramuscularly for five days was suggested as an effective method for proper treatment of bovine actinomycosis.

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