

Fatal Actinobacillosis in a Dairy Buffalo

G. MUHAMMAD, M. SAQIB, M. ATHAR

Department of Veterinary Clinical Medicine and Surgery,
Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

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Abstract

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The present report describes a case of fatal actinobacillosis in a dairy buffalo (*Bubalus bubalis*). Clinical signs included a large multilobular, firm abscess located caudo-ventral to the right ear base, submandibular and prescapular lymphadenopathy, fever and dyspnoea. Haemogram revealed an accelerated erythrocyte sedimentation rate and erythropenia. Provisional diagnosis of actinobacillosis was confirmed by the demonstration of a Gram-negative pleomorphic organism in pus, microscopic examination of a compression preparation of a sulphur granule as well as growth of the organism on blood agar. Intraperitoneal inoculation of pus in guinea pigs was fatal at around 48 hours post-injection. The LD₅₀ of the *Actinobacillus lignieresii* isolate in mice was 10⁴ colony forming unit per milliliter of Hank's balanced salt solution. The animal died 12 hours after the initiation of surgical drainage of the abscess and administration of iodide, antibiotics and steroids. Necropsy findings included interlobular pneumonia, pulmonary abscessation and mediastinal lymphadenopathy that were ascribed to metastatic spread of the organisms to the lungs which probably led to the death of the animal. This case report appears to be the first documentation of fatal actinobacillosis in the dairy buffalo.

Actinobacillosis, Actinobacillus lignieresii, Bubalus bubalis, dairy buffalo

Actinobacillosis is an infectious, chronic, generally non-fatal disease caused by *Actinobacillus lignieresii*. In cattle, it typically involves the tongue and hence is also known as “the Wooden Tongue”. The involvement of other organs is considered to be atypical (Mohanty and Singh 1970; Rebhun et al. 1988; Aslani et al. 1995; Holzhauser and Roumen 2002). The etiologic agent is found normally in the oropharynx and rumen of cattle and sheep (Smith 1990; Songer and Post 2005). The organism is an opportunistic pathogen and it causes chronic pyogranulomatous lesions of the soft tissues of head and neck regions in cows, buffalo, sheep, goats, and horses (Misra and Angelo 1981; Fubini and Campbell 1983; Baum et al. 1984; Kumar and Parihar 1998; Hirsh et al. 2004; Songer and Post 2005). Clinically, the lesions appear as nodules, multiple abscesses, ulcers or draining fistulae. Spontaneous actinobacillosis in the buffalo (*Bubalus bubalis*) is reportedly non-fatal (Akhtar and Cheema 1964; Ahmad et al. 1986). The present report describes a case of atypical, fatal actinobacillosis in a dairy buffalo.

Case Report

An 8-year old, approx. 500 kg Nili-Ravi dairy buffalo in the 4th month of her 5th lactation was presented to the Veterinary Medical Teaching Hospital, University of Agriculture, Faisalabad, Pakistan, for the treatment of a large, lobulated abscess at the base of the right ear and additional swellings cranial to both the right and left shoulders. Anamnesis indicated that the condition began five months previously as a small, walnut-size swelling below the base of the right ear. The swelling gradually enlarged over this period and became lobulated. One of lobules of the abscess located at the ear base broke open about 3 weeks prior to presentation. The animal was treated with a long-acting preparation of oxytetracycline for two days before presentation. The animal was also anorexic for the last couple of days.

Address for correspondence:

Dr. M. Saqib, Department of Veterinary Clinical Medicine & Surgery
University of Agriculture,
Faisalabad 38040, PAKISTAN

Phone: +92(41) 9200161/3110
Fax: +92(41) 9200204
Email: drsaqib_vet@hotmail.com
<http://www.vfu.cz/acta-vet/actavet.htm>

On clinical examination, the buffalo exhibited fever (103 °F; 39.4 °C), mild dyspnoea and halitosis. The most noticeable sign was the presence of a large (33 × 18 cm), warm and firm, lobulated abscess located caudo-ventral to the right ear. This abscess had the contour of four tennis ball-sized lobules as well as a walnut-sized one. The posterior-most lobule located just adjacent to the ramus of the mandible had a small deep sinus that was discharging a mucoid, greyish-white, slightly putrid, non-gritty pus. Right and left prescapular lymph nodes were grossly enlarged to the size of a muskmelon (approximately 18 × 15 cm). The submandibular lymph nodes were also extensively enlarged.

Significant haematological alterations included an accelerated erythrocyte sedimentation rate (120 mm after first hour) and erythropania (4.1 million μl^{-1}). Using a sterile disposable syringe, about 10 ml of greyish-white mucoid pus was aspirated from the dorsal lobule of the abscess located directly below the right ear. Microscopic examination of Gram-stained smears of the pus revealed a Gram-negative organism in isolated coccoid, filamentous and club forms. Culture of this pus on blood agar plates yielded small (1 to 2 mm), raised, greyish-white, non-haemolytic colonies within 24 hours. Gram-stained smears of these colonies revealed small Gram-negative rods. Intraperitoneal (IP) inoculation of 1 ml of pus diluted with an equal volume of saline into two guinea pigs (in accordance with the requirements of laboratory animal use in biomedical research as defined by The Animal Ethics Committee, University of Agriculture, Faisalabad) led to distension of the peritoneal cavity with a turbid serous fluid as well as the guinea pigs' deaths at around 48 hours post-injection.

After the aspiration of pus with a syringe, the dorsal mature pocket of the abscess below the right ear was surgically lanced and about 50 ml of pus was collected in a Petri dish. Careful exploration of this pus revealed only a single whitish-yellow pinhead-sized sulphur granule. After washing in sterile water, this granule was crushed between two microscope slides and Gram stained. Microscopic examination of the stained smears showed Gram-negative bacilli, cocci and short filaments. The combination of clinical signs, cultural and microscopic examination of the pus and a sulphur granule provided the basis for diagnosis of actinobacillosis in the subject of the present report.

The upper-most matured pocket of the lobulated abscess below the right ear was surgically lanced, drained and flushed with a 1% solution of povidone-iodine Pyodine, Brooks Labs., Pakistan). Twelve grams of potassium iodide was dissolved in water and the buffalo was drenched with it. Twenty millilitres of trimethoprim-sulphadiazine (Inj. Tribissen 48%, Glaxo-Wellcome-Beechem, Pakistan) and 10 ml of prednisolone-dexamethasone (Inj. Pre-Dexa, Shinil, Korea) were administered intramuscularly. The buffalo died 12 hours after the institution of therapy. *Post mortem* examination revealed blood-tinged fluid in the thoracic cavity and bilaterally congested lungs with almond-sized abscesses in their anterior lobes. Mediastinal lymph nodes were moderately enlarged. The material aspirated from the lungs abscesses and mediastinal lymph nodes yielded non-haemolytic, small, greyish white, raised colonies after 48 hours of culture on blood agar. The organism was catalase- and oxidase-positive. It fermented fructose, glucose, galactose, maltose, mannose, sucrose, and xylose without gas production. Methyl red, Voges-Proskauer and indole tests were negative. Four different dilutions (10^4 , 10^6 , 10^8 , 10^{10} colony forming unit (cfu) per millilitre of Hank's balanced salt solution) of *Actinobacillus lignieresii* isolate were injected IP into sixteen mice; four mice per dilution. Four additional mice injected IP with 1 ml of Hank's balanced salt solution alone, served as controls. The mice weighed 20-25 g. The injections were made into the left lower abdominal quadrant with a 25-gauge needle. The most dilute inoculum (10^4 cfu/ml) culminated in the death of two of the four mice at 24 and 27 hours post inoculation. The remaining three concentrations (10^6 , 10^8 and 10^{10} cfu/ml) led to the death of all the inoculated mice between 18-21, 9-11.5 and 9-11 hours post inoculation, respectively, whereas all mice in the control group survived. The organism was re-isolated from all mice inoculated with the bacterial suspension.

Discussion

As far as could be ascertained from the literature, the present report appears to be the first record of fatal spontaneous actinobacillosis in the dairy buffalo. In reported outbreaks of actinobacillosis in the buffalo (Akhtar and Cheema 1964; Ahmad et al. 1986), the case fatality rate was zero. Cheema (1965), however, documented that experimental inoculation of *Actinobacillus lignieresii* isolated from the buffalo was fatal in buffalo calves within seven months but not in bovine calves. Respiratory difficulty observed in the subject of the present report may be ascribed to metastatic extension of the disease to the lungs (Cheema 1965; Gill and Balwant-Singh 1977; Carter and Chengappa 1991). Similarly, extensive swelling of submandibular lymph nodes as well as right and left pre-scapular lymph nodes may be attributed to metastatic spread and localization in these glands (Shastri 1983; Aslani et al. 1995; Radostits et al. 2000). Fatality of guinea pigs after intraperitoneal inoculation concurs with the observation of Cheema (1965). The biochemical characteristics of the organism isolated from pus and characterized as *A. lignieresii* were consistent with those described by Lentsch and Wagner (1980).

Actinobacillosis in cattle typically involves the tongue, and the involvement of other organs is considered to be atypical. The disease process in the buffalo of the present report did not affect the tongue. Apart from a solitary report of lingual actinobacillosis in a buffalo (Misra and Angelo 1981), all other reports on actinobacillosis in the buffalo describing sporadic cases (e.g. Panchbhair and Deshpande 1985) or outbreaks of this disease (Akhtar and Cheema 1964; Ahmad et al. 1986) documented only cutaneous involvement without tongue involvement. It may be that contrary to bovine actinobacillosis, bubaline actinobacillosis typically involves the skin and not the tongue. Additional clinical research is clearly warranted to verify or refute this tentative conclusion.

Fatální aktinobacilóza u buvola domácího

Práce popisuje případ fatální aktinobacilózy u buvola domácího (*Bubalus bubalis*). Klinickým nálezem byl velký multilobulární absces, kaudovětrálně od báze pravého ucha, lymfadenopatie (submandibulární a preskapulární mízní uzliny), horečka a dyspnoe. Hematologické vyšetření ukázalo zvýšenou a zrychlenou sedimentaci a erytropenii. Předběžná diagnóza aktinobacilózy byla potvrzena nálezem gram-negativních pleomorfních organismů v hnisu, mikroskopického vyšetření kompresního preparátu a růstu mikroorganismů na krevním agaru. Intraperitoneální aplikace hnisu byla pro morčata fatální kolem 48 hod po aplikaci. Poloviční letální dávka, LD₅₀ izolátu *Actinobacillus lignieresii* byla u myši 10⁴ CFU.ml⁻¹ (colony forming unit) Hankovy soli. Buvol uhynul 12 hodin po zahájení chirurgické drenáže abscesu a aplikaci jodu, antibiotik a steroidů. Při pitvě byla zjištěna interlobulární pneumonie, abscedace v plicích a mediastinální lymfadenopatie, která byla připisována metastatickému šíření mikroorganismu do plic, což pravděpodobně vedlo k uhnutí zvířete. Tento případ je pravděpodobně první popsána fatální aktinobacilóza u buvola domácího.

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