

Case report**Use of ultrasound in a case of Actinomyces in a Holstein Friesian cow**

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Abstract

Actinomycosis is a sporadic disease of cattle, caused by *Actinomyces bovis*. In presence of oral injuries or openings *Actinomyces bovis* could penetrate tissues and infect maxilla or mandible bones. A Holstein Friesian cow was presented for inappetence, weight loss and a left-sided mandibular swelling. A unilateral swelling was visible on the left jaw as a ventro-lateral and circular in shape. A fine-needle aspiration was collected. Ultrasonography examination was performed. Bacteriologic analysis was negative. Due to the history, Actinomycosis diagnosis was suggested and the cow was culled. Pathological examination revealed lesions indicative of chronic pyogranulomatous mandibular osteomyelitis with intralesional colonies of Gram-positive bacteria, consistent with infection due to *A. bovis*. The importance of an early diagnosis was underlined. The bacterial culture is not always easy to perform and is usually time-consuming. In case of suspected Actinomycosis, the ultrasonographic findings may orient the clinician decision to start adequate and earlier therapy.

Key words: *Actinomyces bovis*, dairy cows, lumpy jaw, ultrasonographic evaluation

30 Introduction

31 Actinomycosis (lumpy jaw) is a sporadic disease of cattle, caused by *Actinomyces bovis*, a Gram
32 positive, non-encapsulated, filamentous organism which is normally part of the oral and
33 digestive tract flora of the ruminant species (Smith, 2015; Peek *et al.*, 2018).

34 In presence of oral injuries or openings, caused by coarse fibrous feeds, hard plant awns or
35 gingival and dental disease, *A. bovis* could penetrate tissues and infect maxilla or mandible
36 bones (Smith, 2015). The bacterium can also penetrate across contaminate skin wounds and
37 around the alveoli of the teeth (Peek *et al.*, 2018).

38 *A. bovis* can cause severe osteomyelitis of the mandible, or less frequently of the maxilla (Smith,
39 2015), determining an increased culling rate in the bovine herd. Clinically, actinomycosis
40 appears as a warm and painless swelling area on mandible, without other clinical signs. The
41 swelling area slowly increases and becomes harder and usually painful at the digital palpation.
42 The osteomyelitis caused by this bacterium could lead, in untreated cows, to the deformation
43 of infected bones with important problems to the anchored teeth (Peek *et al.*, 2018).

44 Ultrasonography of the soft tissues is usually indicated when a soft tissue swelling is palpated
45 or identified radiographically. High frequency ultrasonography is considered a useful tool for
46 the evaluation of the skin and other superficial soft tissues affected by focal or diffuse
47 conditions (Rossi, 2011).

48 In veterinary medicine, computed tomography and radiographic examination are considered the
49 gold standard to fully characterize osseous abnormalities of the head, but they have limited
50 availability in the field. Since many soft tissue structures of the head are superficially located
51 and easily accessible, the ultrasonography is considered a valuable tool in field settings for the
52 diagnosis of these diseases. Although there are many publications describing the use of
53 ultrasound for examination of specific abnormalities or focused regions of the equine head
54 (Rodriguez *et al.*, 2007; Ali, 2000), the same has not yet been described in the bovine.

55 **Materials and methods**

56 A 3-years-old first lactating Holstein Friesian cow was presented for inappetence, weight loss
57 and a left-sided mandibular swelling. At the admission time, the cow was 273 days in milking
58 (DIM) and was not pregnant. The cow belonged to a dairy farm consisting of approximately
59 sixty lactating cows bred in free housing system. Lactating cows were fed with total mixed
60 ration (TMR) two times per day.

61 The farmer reported that the swelling area appeared 2 months before and have been increasing
62 in size, slowly but progressively. The owner also reported a similar case in a lactating cow six
63 months before, which was slaughtered for fertility problems. A blood samples were collected
64 from the jugular vein for running a complete cell blood count (CBC) and biochemistry analysis.
65 Ultrasonography (Z5 Vet, Mindray, China) and X-Ray (HF100 M, Gierth, Germany; Software
66 program Horos, Horosproject.org, USA) examinations of the swelling area were also
67 performed. A convex probe (4.5 MHz) was used. The whole part was scanned in longitudinal
68 and transverse view for evaluating the content, the edges and the layers involved. The
69 radiographic examination was performed using a laterolateral (Kv 66 and mAS 8) and a
70 ventrodorsal (Kv 73 and mAS 8) view. Finally, samples for the bacteriological examination
71 were collected aseptically from the same site by the fine-needle aspiration.

72 **Results and discussion**

74 At the admission time the cow appeared alert and responsive. A unilateral swelling was visible
75 on the left jaw as a ventro-lateral, circular in shape and about 15 cm of diameter in size mass.
76 No cutaneous wound or fistula were visible, and the swelling area was hard, warm, immovable
77 and painful on digital palpation (Fig. 1). The cow was in poor general condition, with a BCS of
78 2.25/5 and showed a profound salivation and inappetence. The cow presented a slightly reduced
79 rumen contractions and feces with not digested materials. These findings were probably caused

80 by the reduced capacity of chewing the food due to the swelling area. The hematologic and
81 biochemistry findings were normal.

82 The ultrasonography of the swelling revealed a circular and well-defined structure in the
83 subcutaneous layer, delineated by a thick, hyperechoic peripheral wall. The content of this
84 structure had a mixed echogenicity, mainly hyperechoic (Fig. 2). The ultrasonographic image
85 was suggestive of an abscess, a granuloma or an organized hematoma. At the radiographic
86 examination a wide, smooth, continuous and fusiform periosteal reaction on the ventral edge of
87 the left mandible's body was found. Multiple, circular and radiolucent areas with clear edges
88 were on the body of the mandible. These alterations were suggestive of osteomyelitis.

89 Bacteriological examination was performed according to protocol suggested (Militerno, 2008)
90 and it came negative. The most important differential diagnosis included Actinomycosis,
91 osteomyelitis induced by other pathogens and neoplasia. Due to the poor prognosis, the farmer
92 decided to cull the cow. For completeness of the case, the head of the cow was taken from the
93 slaughterhouse and submitted for pathology. On cut section, there were numerous encapsulated
94 granulomas, up to 5 cm in diameter, in the subcutaneous tissues and mandibular muscles (Fig.
95 3a). Small abscesses and sequestered trabeculae of affected bone were also found within the
96 lesions, accompanied by extensive proliferation of granulation tissue. The abscesses contained
97 numerous yellowish particles of less than 1 mm in diameter, interpreted as Sulphur granules.
98 After removal of all soft tissues, necrosis and proliferation of bone was observed giving rise to
99 an enlarged, honeycomb appearance of the body of the mandible (Fig. 3b).

100 Histologically, most of the mandibular bone was replaced by fibrovascular tissue surrounding
101 many small abscesses with granules composed of large bacterial colonies. Granules were
102 surrounded by radiating eosinophilic clubs, interpreted as Splendore-Hoeppli material.

103 The lesions were indicative of chronic pyogranulomatous mandibular osteomyelitis with
104 intralesional colonies of Gram-positive bacteria, consistent with infection due to *A. bovis*.

105 Given the farmer history, a herd visit was also performed. At the time of the inspection, all the
106 animals were in good general conditions and the milk production was in line with the average
107 reference production of a dairy herd. At food inspection of the lactating group, a very fibrous
108 hay, composed mostly by hard plant awns and stickers, was found in the TMR. A complete
109 report was written and sent to the farmer a week later.

110 The diagnosis of Actinomycosis in cows is mainly based on radiographic and bacteriological
111 examination (Peek *et al.*, 2018). The involvement of *A. bovis* was not confirmed by the
112 bacteriologic analysis, however, many authors reported that a conclusive diagnosis made by
113 bacterial culture is rare to achieve (Smith, 2015; Peek *et al.*, 2018).

114 In the present case, the ultrasonography was easy to perform in field condition, not time
115 consuming and the image was clear and easy to evaluate. The ultrasonographic examination
116 could be also made by a reproductive linear probe which represents an essential tool for the
117 bovine practitioner. The imaging recorded, along with a proper history, should immediately
118 suggest a case of Actinomycosis and encourage a conclusive diagnosis through the
119 bacteriological examination.

120 Actinomycosis is usually a sporadic disease, but it could be an epidemic or endemic herd
121 problem, especially related to the presence of an increasing risk of oral injuries that lead to *A.*
122 *bovis* penetration into the tissues (Militerno, 2008; Smith *et al.*, 2015). In the present farm, the
123 occasionally use of a fibrous and awful quality hay could have played a key role in the
124 development of Actinomycosis.

125 Given the complexity of the therapy and costs, preventive actions are mandatory. The owner
126 was strongly encouraged to use always excellent quality hay, avoiding the use of forage rich in
127 thorns, stickers and hard plant awns.

128 Due to the importance of an early diagnosis of *A.bovis* mandibular osteomyelitis, ultrasound
129 could provide supportive evidence of *A.bovis* infection in this case, especially important since
130 culture of *A. bovis* is unreliable. Ultrasound is also easier and safer to perform than radiography.

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149 **Figure legends**

150 **Figure 1** Swelling area on the left jaw visible as a ventro-lateral, circular in shape and about 15
151 cm of diameter in size mass in a 3-years-old first lactating Holstein Friesian cow.

152 **Figure 2** Ultrasound of the swelling area visible on the left jaw of a 3-years-old first lactating
153 Holstein Friesian cow. The content of this structure had a mixed echogenicity, mainly
154 hyperechoic. The ultrasonographic examination was made using a convex probe (4.5 MHz) (Z5
155 Vet, Mindray, China).

156 **Figure 3a** Ventro-lateral aspect of the left mandible in a 3-years-old first lactating Holstein
157 Friesian cow. Numerous granulomas in the subcutaneous tissues and muscles of the mandibular
158 region.

159 **Figure 3b** Left mandible of a 3-years-old first lactating Holstein Friesian cow. Honeycomb
160 appearance of the mandibular body caused by extensive necrosis and concurrent proliferation
161 of bone tissue.

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Figures

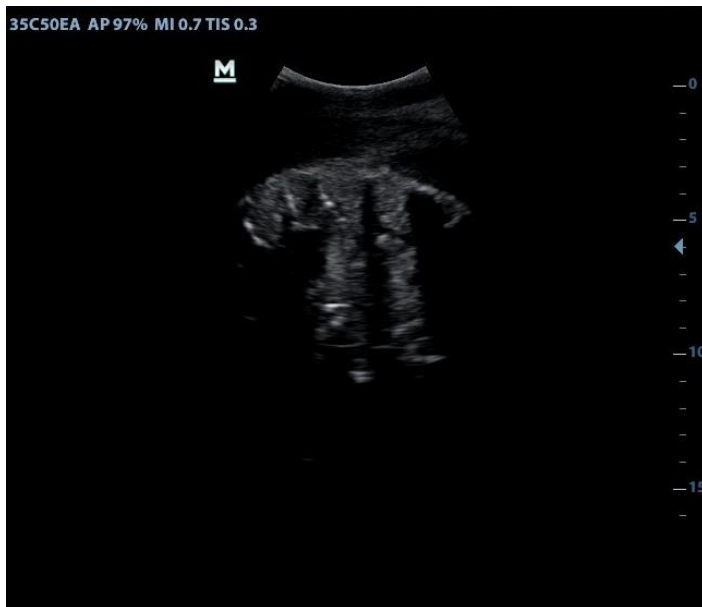


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Figure 1.

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Figure 2.

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Figure 3a-b.