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Scientific Report

Occurrence of purulent mandibular and maxillary osteomyelitis associated with *Pseudomonas aeruginosa* in a sheep flock in south-west of Iran

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Summary

This report describes an outbreak of purulent mandibular and/or maxillary osteomyelitis caused by *Pseudomonas aeruginosa* in a sheep flock located in the Khuzestan province, Iran. Jaw bones of almost 100 out of 500 mature sheep in a flock became severely deformed with a variably sized firm swelling, without any signs of inflammation in the surrounding soft tissues. The affected animals showed anorexia, depression, swelling of the mandibular and/or maxillary area, loss of cheek teeth and poor body condition. These animals were gradually culled in a period of 3 months. Postmortem examination showed a hard swelling of jaw bones with dirty greenish pus that filled alveolar molar teeth cavities. Histopathologic findings revealed necrotic areas surrounded by mixed population of inflammatory cells with exuberant fibrosis around some area of the lesions and irregular trabeculae of woven bone. In bacteriology, pure culture of *P. aeruginosa* was isolated from all of 7 sampled sheep. Based on clinical examination, radiography, histopathological features and bacteriology, the lesions were diagnosed as chronic suppurative osteomyelitis caused by *P. aeruginosa*. According to bacteriological results, the likely source of bacterial infection in this study was drinking water.

Key words: Mandibular, Maxillary, Osteomyelitis, *Pseudomonas aeruginosa*, Sheep

Introduction

Osteomyelitis is an inflammation of bone, localized or generalized, due to a pyogenic infection (Blood *et al*., 2007). Inflammation of bone is uncommon in farm animals except when infection is introduced by traumatic injury or by the hematogenous route (Constable *et al*., 2017). Osteomyelitis affects all species but is most common in young animals. The specific causes of osteomyelitis in animals include spinal abscesses from tail docking, and omphalophlebitis or septicemias which result in infectious arthritis, meningitis, and also osteomyelitis (Ogilvie, 1998). Numerous bacteria such as *Trueperella pyogenes*, *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. cause osteomyelitis in animals (Benito-Pena *et al*., 2010). Mandibular osteomyelitis has been reported frequently in animals. The term "lumpy jaw" refers to the disease in cattle and occasionally other animals in which *Actinomyces bovis* is the causative agent (Benito-Pena *et al*., 2010). Antunes *et al*. (2012) reported the occurrence of unusual jaw infection caused by *A. bovis* in a sheep. Lumpy jaw is widespread in wild sheep of North America (Hofes and Bunch, 2001). However, numerous other bacteria such as *T. pyogenes*, *Fusobacterium necrophorum*, *Staphylococcus aureus*, and *Streptococcus* spp. are isolated from mandibular osteomyelitis in wild sheep (Glaze *et al*., 1982; Hofes and Bunch, 2001). Hoefs and Bunch (2001) determined the prevalence and geographic distribution of lumpy jaw in wild sheep of North America. There are numerous pseudomonads but only one species *Pseudomonas aeruginosa* is of considerable pathogenic significance in animals (Carter and Blackwell, 2004). The present case report describes occurrence of purulent mandibular and maxillary osteomyelitis associated with *P. aeruginosa* in sheep in Khuzestan province, in the south-west of Iran.

Case history

In September 2013, three adult ewes (3-5 years of age) with a history of anorexia, mandibular and/or maxillary deformity, difficulty in mastication and loss of weight were presented to the Teaching Veterinary Hospital of Shahid Chamran University of Ahvaz, Iran. These animals belong to a flock of 500 sheep that grazed extensively around Hamidieh town, located in the Khuzestan province, in the south-west of Iran. Clinical examination of presented animals followed by initial visit to the farm revealed anorexia, depression, variably sized firm swelling of the mandible and/or maxilla in all

affected animals (Fig. 1) with purulent discharge in some animals, loss of molar teeth with impacted forage between cheek teeth and poor body condition. Affected animals were culled in a period of about 3 months. At the time of our visit to the farm, about 100 ewes (20%) were affected and 25 ewes had been culled. Empirical treatment by local veterinarians with oxytetracycline and potentiated sulfonamides with trimethoprim had been ineffective. Swab samples or aspirated material were taken from lesions. From presented animals and 4 others in the herd, blood was taken from jugular vein and sampling of the lesions with swabbing or aspiration was performed. Also, drinking water of affected herd was sampled. The samples were inoculated on blood and McConkey agar and incubated aerobically at 37°C for 24-48 h. Characterization of the isolated bacteria was carried out with the use of standard methods (Markey *et al*., 2013). The antibiotic susceptibility test of isolated bacteria was performed by the agar disk diffusion method. All of the submitted sheep to the Teaching Veterinary Hospital were presented to radiographic evaluation and two more cachectic animals were euthanized for postmortem examination. Radiography was performed in dorsoventral and lateral (slightly oblique) views. Also, after postmortem examination, tissue samples were fixed in 10% natural buffered formalin and decalcified routinely in EDTA. The samples were embedded in paraffin wax, sectioned (5 µm) and stained with hematoxylin and eosin.

Fig. 1: Photograph showing bony swelling of ventral surface of the left mandible

Results

Results of complete blood count were unremarkable. In lateral view of radiography, multiple well defined radiolucent osteolytic areas were seen in mandibular bone which some of them were around mandibular root of molar teeth (Fig. 2). Some of osteolytic lesions had sclerotic margins. Ventral border of the mandible near the osteolytic lesion was thin. Osteogenesis was not seen. Based on history and clinical findings, osteomyelitis was final radiographic diagnosis.

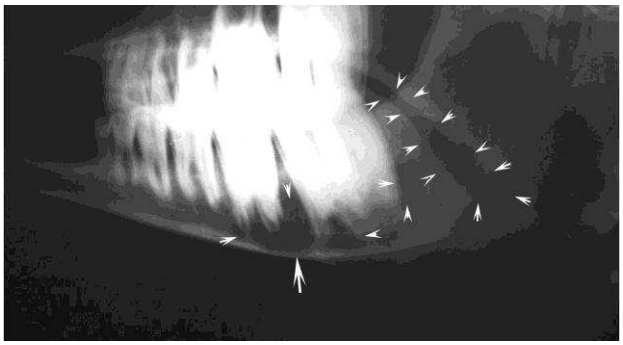

Fig. 2: Right lateral (slightly oblique) radiograph of head of a sheep. Osteolysis is seen with or without sclerosis (arrow heads). Ventral border of mandible is thin (large arrow)

Postmortem examination revealed a hard swelling of jaw bones. In the cut surface of the swelled area, dirty greenish pus that filled alveolar molar teeth cavities and adjacent lytic bone tissue was noted. Microscopically the necrotic areas were surrounded by mixed population of inflammatory cells including neutrophils, macrophages, lymphocytes, plasma cells and also few fibroblasts. Exuberant fibrosis around some area of the lesions and irregular trabeculae of woven bone were also noted (Figs. 3 and 4). Based on histopathological features, the lesion was diagnosed as chronic suppurative osteomyelitis.

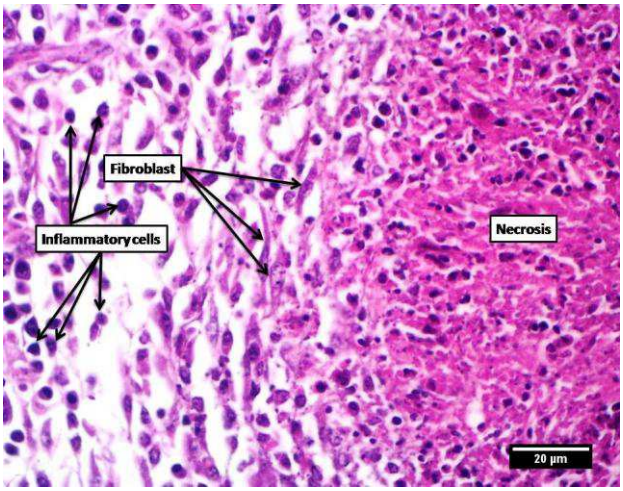

Fig. 3: Border of the lesion showing necrotic tissue that is surrounded by few fibroblasts and mixed inflammatory cells (H&E)

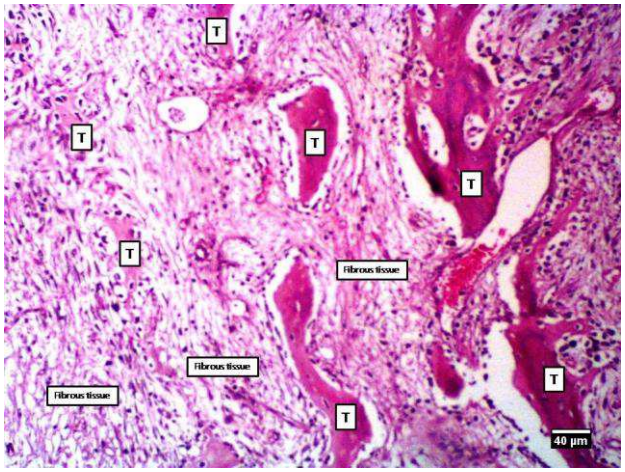

Fig. 4: Irregular trabeculae of woven bone (T) and fibrous tissue that filled inter-trabecular spaces (H&E)

Pure *P. aeruginosa* was isolated from the lesions of animals and drinking water. The antibiotic sensitivity test showed that the isolated bacteria were sensitive to ciprofloxacin, gentamycin and amikacin and resistant to nitrofurantoin, nalidixic acid, sulfamethoxazole, cefixime and cephalothin.

Discussion

Mandibular osteomyelitis has been reported frequently in animals (Benito-Pena *et al*., 2010), but it is a rare condition in small ruminants (Seifi *et al*., 2003). Also, mandibular and maxillary osteomyelitis in sheep caused by *P. aeruginosa* is a rare condition and there are very few reports in this regard (Benito-Pena *et al*., 2010; Amorim *et al*., 2011). The bony lesions observed in the present study frequently resulted in culling of the animals due to tooth loss, accompanying pain, and decreased food intake. The infected sheep flock in this report was grazed on an arid rangeland with poor vegetation that mainly included thorn bushes. Water consumption was from an irrigation canal, sometimes with running water but usually with remaining water at the bottom. It is believed that, possibly sustained injury of the oral cavity mucosa caused by abrasions from grazing on rough forage and ingestion of plant awns, followed by invasion of organisms present in the environment or oral cavity provide mandibular and maxillary osteomyelitis (Brookins *et al*., 2008). Benito-Pena *et al*. (2010) reported purulent nasomaxillary and mandibular osteomyelitis in sheep and isolated *P. aeruginosa* as a causative organism. Outbreak of mandibular abscess by *P. aeruginosa* in sheep was reported by Amorim *et al*. (2011) as the misuse of dosing guns for anti-helminth in sheep was considered as a destructive agent in the oral cavity. In a captive herd of red kangaroos, mandibular and maxillary osteomyelitis caused by *Pseudomonas* spp. has been reported (Brookins *et al*., 2008). *Pseudomonas aeruginosa* is an opportunistic pathogen and generally requires a defect or a change in the natural defense of the body to cause an infection (Gyles *et al*., 2010). Its natural habitat is water, soil, plants (Gyles *et al*., 2010), and it may also be found on the skin and mucous membranes and in faeces (Carter and Blackwell, 2004). It has been shown that contaminated water used for premilking udder washing may cause pseudomonas mastitis in cows (Erskine *et al*., 1987). Las Heras *et al*. (1999) reported that water used to clean milking equipment system was the most likely source of acute ovine mastitis associated with *P. aeruginosa*. Isolation of the organism from skin, ear, udder and lung infections have been reported (El-Sukhon, 2002; Watson *et al*., 2003; Leitner and Krifucks, 2007). In animals, a variety of diseases are caused by *P. aeruginosa*, which includes wound infections in all species, urinary tract infections and chronic purulent otitis externa in dogs, ulcerative keratitis in dogs and horses, and dermatitis (fleece rot) in sheep (Gyles *et al*., 2010). In one case, several ewes developed severe purulent rhinitis and otitis externa/media after being showered with a wash that had previously been used on another group of ewes with dermatitis (Watson *et al*., 2003). In the current case, according to the results of microbial culture and based on the literature in this regard that is mentioned above, water was considered as the potential source of infection. Due to the poor prognosis of osteomyelitis in large animals and results of low efficiency of treatment with gentamicin that was performed on a few cases, culling of infected animals and supplying clean water source and avoidance of mucosal trauma by ingestion of soft feed was recommended, but follow up to one year did not show a significant change in the herd status because of the owner's negligence. Treatment with antimicrobial agents is generally unsuccessful because *P. aeruginosa* is an intrinsically multidrug-resistant bacteria and the poor vascularity of the affected solid bone (Constable *et al*., 2017).

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Conflict of interest

The authors declare that they have no conflicts of interest.

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