CASE REPORT

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Diagnosis and surgical management of acanthomatous ameloblastoma in a 5-month-old female Boerboel puppy

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Abstract
A five-month-old female Boerboel puppy presented with a rapidly expanding fibrous growth around the canine tooth of the right maxilla was diagnosed as having acanthomatous ameloblastoma based on findings from skull radiography, cytology of fine needle aspiration, biopsy and histopathology of resected growth. Lateral radiograph of the skull revealed moderate osteolysis of the maxilla with presence of un-erupted maxillary canine teeth. Cytology of fine needle aspirate from the growth showed clusters of epithelial cells that were hyperchromatic and showing palisade arrangement. The mass was surgically resected using diathermy instrument following general anaesthesia using combination of intramuscular injection of xylazine (0.5mg/kg), and intravenous injection of propofol (4mg/kg). Post-operative management included Ibuprofen suspension (100mg) administered for 3-days, intravenous vincristine (0.5mg/m²) once weekly and oral cyclophosphamide (50mg) was administered twice weekly for six weeks. Histopathology of the growth showed odontogenic epithelium embedded in pulp ectomesenchyme. Features of odontogenic epithelium include palisading epithelium with anti-basilar nuclei and centrally located cells having features of stellate reticulum. This is probably the first reported case of acanthomatous ameloblastoma in a Boerboel puppy that was successfully managed.

Keywords: Acanthomatous, Ameloblastoma, Boerboel-puppy, Chemotherapy, Odontoid

Introduction
Oral tumours are the fourth most common neoplasm in dogs accounting for 7% of all canine tumours (Saffari et al, 2019). They either originate from oral mucosa, dental root, and periodontal tissue or from maxillary, mandibular, incisive or palatine bones (Huang et al., 2019). They are usually not discovered in early developmental stage until they become visible or when symptoms of oral illness are observed (Malmberg et al., 2017). Symptoms commonly associated with oral cavity tumours include halitosis,
ptyalism, exophthalmos, facial swelling, weight loss, epistaxis, bloody oral discharge, dysphagia or pain on opening the mouth (Huang et al., 2019). Commonly reported tumours of the oral cavity in dogs include melanoma, squamous cell carcinoma, fibrosarcoma, canine acanthomatous ameloblastoma, and peripheral odontogenic fibroma (Liptak & Withrow, 2007).

Oral tumours are classified as either odontogenic or non-odontogenic based on their origin and histology (Huang et al., 2019). Odontogenic tumours are derived from ectomesenchymal and/or epithelial tissue responsible for tooth formation (Goldschmidt et al., 2017). They include acanthomatous ameloblastoma, odontoma, and odontogenic fibroma (Huang et al., 2019). Non-odontogenic oral tumour could occur in any other part of the oral cavity and include melanoma, Squamous cell carcinoma, fibrosarcoma, and osteosarcoma (Liptak & Withrow, 2007).

Canine acanthomatous ameloblastoma (CAA) is a benign locally aggressive odontogenic tumour of the jaw arising from periodontal ligament and capable of invading surrounding tissue of the mandible or maxilla (Liptak & Withrow, 2007). It is the most common canine odontogenic tumour with prevalence ranging from 19.1% to 35% (Goldschmidt et al., 2017). CAA is believed to arise from odontogenic epithelium in the gum, residual dental lamina, or basal epithelial cells of the oral mucosa (Huang et al., 2019). It is a locally invasive and destructive tumour which often results in alveolar bone lysis, loss of teeth and rapid spread to cancellous bone (Malmberg et al., 2017). It has been reported in Golden Retriever, Labrador retriever, German Shepherd, Pitbull, Samoyed, Beagles and other dog breeds (Malmberg et al., 2017). There is no reported sex and breed predisposition (Liptak & Withrow, 2007). However, some studies have reported more prevalence in male than in female (Malmberg et al., 2017; Saffari et al., 2019). Age incidence of CAA in dog varies and ranges between 3-13 years (Malmberg et al., 2017; Saffari et al., 2019) with mean age of 7.5 years. Canine oral tumours are rarely reported in Nigeria and there is dearth of information on acanthomatous ameloblastoma in dogs. This report presents a case of acanthomatous ameloblastoma in a five-month-old female Boerboel puppy.

Case Management
A five-month-old female Boerboel puppy was presented with a growth on the gingival tissue of the right maxilla between the canine and premolar teeth (Plate I). The owner had first observed the growth six weeks before presentation and reported that it had rapidly increased in size. It was also reported that the growth had interfered with the feeding of the puppy. Clinical examination revealed that the physiological parameters of the dogs were within normal range. The submandibular lymph nodes and other peripheral lymph nodes were not enlarged. The right upper lip was raised as a result of the expanding gingival growth measuring about 3cm in diameter. There was moderate halitosis. The growth appeared fibrous with a somewhat mulberry surface and bled when firm pressure was applied on it. In addition, the...
Table 1: Complete blood count and selected serum biochemistry parameters of a five-month-old Boerboel puppy with odontoid ameloblastoma

| Parameters                  | Patient value before surgery | Patient value 3 days post-surgery | Patient value 1-week post treatment | Reference value |
|-----------------------------|-----------------------------|-----------------------------------|-------------------------------------|-----------------|
| PCV (%)                     | 43                          | 35                                | 42                                  | 37-55           |
| WBC (×10^9/L)               | 6.5                         | 18                                | 17.9                                | 6-17            |
| Neutrophil (%)              | 68                          | 67                                | 65                                  | 60-70           |
| Lymphocyte (%)              | 28                          | 32                                | 30                                  | 12-30           |
| Monocyte (%)                | 03                          | 01                                | 03                                  | 3-10            |
| Eosinophil (%)              | 01                          | 00                                | 01                                  | Rare            |
| Basophil (%)                | 00                          | 00                                | 01                                  | 2 – 10          |
| Alkaline Phosphatase (IU/L) | 105                         | -                                 | -                                   | 17 – 111        |
| Urea (mg/dL)                | 16.4                        | -                                 | -                                   | 10 – 32         |
| Creatinine (mg/dl)          | 0.8                         | -                                 | -                                   | 0.6 - 1.4       |
| Sodium (mmol/L)             | 145                         | -                                 | -                                   | 142 – 150       |
| Potassium (mmol/L)          | 4.0                         | -                                 | -                                   | 3.8 – 5.4       |
| Chloride (mmol/L)           | 110                         | -                                 | -                                   | 105 - 116       |
| Bicarbonate (mmol/L)        | 22                          | -                                 | -                                   | 15 – 25         |

Source of reference value - Aiello & Moses (2013)

Canine tooth around the growth did not erupt. Apart from the oral lesions, the puppy was apparently in good health. Based on the gross appearance and location of the mass, a tentative diagnosis of odontogenic tumour was made.

Both right and left lateral, as well as cranio-caudal radiographs of the skull were obtained using digital x-ray machine (Mobillet XP; Siemens Healthcare GmbH, Germany). Radiographic exposure was made using 70KvP and 12mA. In addition, stationary x-ray grid was used to prevent scattered radiation. In addition, fine needle aspiration biopsy of the growth was obtained for cytology, while blood was obtained for full blood count and selected blood chemistry. The right lateral radiograph of the skull (Plate II) showed moderate osteolysis of the maxilla with infiltrating soft tissue opacity. The tumour in this dog was considered to be in stage T2 based on the size of the oral mass. Two canine teeth that failed to erupt were observed in the maxilla dental arcade and appear to originate from the nasal turbinate. The cytology (Plate III) of the fine needle biopsy showed clusters of epithelial cells that were hyperchromatic and showing palisade arrangement. In addition, there were numerous erythrocytes and leukocytes. The results of the full blood count and serum chemistry were essentially normal (Table 1). Based on the radiographic and cytological findings, a diagnosis of acanthomatous ameloblastoma was made and the dog was scheduled for surgical excision of the growth.

Food was withheld from the dog for 12 hours prior to surgery. The dog was premedicated with 3mg/kg intramuscular injection of 5% tramadol (Tramadol®, Gland Pharma, India) and one hour later with a combination of 0.04mg/kg intramuscular injection of 0.1% atropine sulphate (Atocan®, Sishui Xierkang Pharma, China) and 0.5mg/kg intramuscular injection of 2% Xylazine Hydrochloride (Xylased, Bioweta, Ivanivice, Czech Republic). Venous access was secured through the cephalic vein and circulating blood volume was maintained with Lactated Ringers solution (Unihart® Unique Pharmaceutical Ltd, Sango Ota, Ogun State Nigeria) at the rate of 5 mL/min. Anaesthesia was induced with a loading dose of 4mg/kg intravenous injection of 1% propofol (Hyprovan 200®, Celon Laboratories PVT LTD, Telangana, India) and maintained with constant infusion of propofol at the rate of 0.12mg/kg/min. Additionally, local analgesia of the gum was achieved using 10% lignocaine spray (Xylocaine®, AstraZeneca, Cambridge, UK).

Following anaesthesia, the dog was placed on sternal recumbency with the neck slightly elevated with a soft pillow. The oral cavity was first rinsed with triclorophynyl-methylidosalicyl solution (TCP®, Chemineau Laboratories, France) diluted in normal saline. Thereafter, the growth was surgically excised using bipolar diathermy instrument (MeCan Medical Ltd Guangzhou Guangdong, China). Extensive excision was performed until clear margins of gingival
Plate III: Cytology of fine needle gingival growth aspirate from a five-month-old Boerboel puppy showing clusters of hyperchromatic epithelial cells with palisade arrangement (black arrows) stained with H&E. x400

Plate IV: Immediate post-surgical view of surgically excised gingival mass in a five-month-old female Boerboel puppy

Plate V: Five-month-old female Boerboel dog three weeks after surgical excision of gingival mass

Plate VI: Histologic section from a five-month-old Boerboel dog with acanthomatous ameloblastoma showing odontogenic epithelium embedded in pulp ectomesenchyme (black arrows). H&E. x400

Tissues were obtained (Plate IV). After surgical excision of the growth, haemorrhage was controlled using digital pressure and the oral cavity was rinsed again with the TCP solution again. Topical salicylate cream (Bonjela®, Reckitt Benckiser, UK) was applied to the wound surface and the dog was monitored till recovery. Following recovery, the mouth was rinsed daily with diluted TCP and Bonjela cream applied on the wound surface. Oral cefadroxil monohydrate suspension (Duricef®, GlaxoSmithKline Pharmaceuticals, Lagos, Nigeria) was administered for five days at the rate of 25mg/kg. In addition, 100mg of ibuprofen suspension (Reprofen®, Reals Pharmaceuticals, Lagos, Nigeria) was administered for three days. Also, about 3 mL blood was taken from the cephalic vein for full blood count three days post-surgery as well as one week after the administration of last chemotherapy (Table 1). Adjunct chemotherapy using intravenous vincristine (Excristin®, Zuvius Life Sciences LTD; Mumbai, India) once weekly at the rate of 0.5 mg/m² and 50mg of oral cyclophosphamide (Cytoxan®, Bristol-Myers Squibb Company, UK) administered twice weekly were given for six weeks. The dog was re-examined at 3 and 6 weeks post-surgery. Examination revealed that there was no lesion at the site suggestive of tumour regrowth and fibrous tissue has filled the wound surface (Plate V).

The resected gingival mass was fixed in 10% formalin for histopathology. Histologic section (Plate VI)
revealed odontogenic epithelium embedded in pulp ectomesenchyme. There were proliferating cells that showed no cellular atypia, and mitotic figures were uncommon. Most of the basal cells of the epithelium were hyperchromatic, columnar and arranged in a palisading form. The nuclei of the basal cells were located at the distal ends of the cells and they exhibited cytoplasmic vacuolations. There were also foci of epithelial cell cords consisting of basal and prickle cells with prominent intercellular bridges. The cells enclosed by the basal cells were spindle-shaped and generally closely or loosely packed together with blood vessels containing red blood cells and surrounded by lymphocytes, plasma cells and macrophages.

Discussion
This report presents a case of acanthomatous ameloblastoma in a five-month-old female Boerboel puppy. The age incidence of acanthomatous ameloblastoma reported ranged between 3-13 years (Malmberg et al., 2017; Saffari et al., 2019) with mean age of 7.5 years. Three cases were reported in young dogs between the ages of 4 – 8 months (Huang et al., 2019). The dog in this report was five-month-old. The early diagnosis of the tumour in this dog might be associated with the rapid growth of the tumour which resulted in facial swelling and interference with feeding. Although, there is no sex or breed predilection for the tumour, most reported cases were in male dogs. The dog in this report was a female Boerboel dog and this perhaps may be the first report of this tumour in Boerboel dogs.

Radiography and histopathology have been most common method of diagnosing acanthomatous ameloblastoma (Malmberg et al., 2017). However, computed tomography or magnetic resonance imaging are being employed in staging the tumour prior to surgical or radiation treatment to accurately determine the extent of the primary tumour (Liptak & Withrow, 2007). In this dog, diagnosis was made using a combination of radiography, cytology of fine needle aspirate and the histopathology of the resected tissue. The use of fine needle aspiration cytology in the diagnosis of odontogenic tumours has not been well reported (Chandavakar et al., 2014). The features of odontogenic epithelium include palisading epithelium with antibasilar nuclei and centrally located cells with features of stellate reticulum (Huang et al., 2019). The palisading arrangement of the ameloblast-like epithelial cells is diagnostic of ameloblastoma and can be a fast, economical and less invasive method of diagnosis when a presumptive diagnosis is made.

Odontogenic tumours are locally aggressive; thus, it is important to stage the tumour once the histologic or cytologic type of the tumour is determined. The primary tumour should be staged using the World Health Organization classification system for tumours of the oral cavity (Mayer & Anthony, 2007). Computed tomography or magnetic resonance imaging is often recommended prior to surgical or radiation treatment to accurately determine the extent of the primary tumour, while magnetic resonance images is reported to provide more accurate assessment of oral tumour margins in soft tissue and bone than computed tomographic (Liptak & Withrow, 2007). Four stages of canine oral tumour are recognized namely T0 to T3 with two variants of stages T1 to T3 (Mayer & Anthony, 2007). The tumour in this dog can be considered to be in stage T1b based on the size of the oral mass (about 3cm in diameter) fixation to tissue and the radiographic evidence of bone lysis in the maxilla suggestive of bone involvement. Due to the highly infiltrative behavior of this tumour, complete radical surgical excision alongside mandibulectomy or maxillectomy depending on the extent of bone infiltration by tumour remains the gold standard for the treatment of acanthomatous ameloblastoma (Saffari et al., 2019). Rim excision technique in which the ventral cortical bone of the mandible or the most dorsal portion of the maxillary or incisor bone remains intact while the tumour, surrounding teeth, and periodontal structures are removed have also been described (Murray et al., 2010). The technique helps to improve dental occlusion, cosmetic appearance and prevents tumour recurrence (Murray et al., 2010). In this case, extensive surgical resection without maxillectomy was used in order to preserve the maxilla because of the age of the dog. Owing to the fact that it may not be possible to remove all tumour cells surgically due to the location or size of a tumour, radiation therapy using orthovoltage radiotherapy has been indicated for dogs with tumours that are not curable with surgery alone (Mayer & Anthony, 2007). However, due to lack of facilities for radiotherapy, adjunct chemotherapy with a combination of cyclophosphamide and vincristine was administered for six weeks. Although, there are no previous report of the use of cyclophosphamide and vincristine in the management of acanthomatous ameloblastoma in dogs, uses of intralesional bleomycin have been reported (Kelly et al., 2010). Finally, the prognosis for acanthomatous ameloblastoma is good if the tumour is diagnosed early and there is complete excision of the tumour.
High recurrence rate was reported in marginally excised tumour (Goldschmidt et al., 2017). Tumour staging can be used in determination of prognosis with dogs that had stage T3 tumours having a 7.9 times higher risk of tumour recurrence than dogs with T1 tumours (Huang et al., 2019). Tumour recurrence was reported in 7 of 39 dogs with acanthomatous ameloblastoma (Mayer & Anthony, 2007). The dog in this case has not been observed with any evidence of tumour recurrence six months after surgical excision and adjunct chemotherapy, even though this period may be considered to be short for tumour recurrence to develop.

**Conflict of interest**
The authors declare no conflict of interest.

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