Scientific Report

Clinicopathological investigation of nutritional osteodystrophia fibrosa in a flock of young stall-fed goats

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Abstract

Background: Osteodystrophia fibrosa (ODF) is a metabolic disorder affecting the skeletal system, causing progressive loss of calcified bone mass and its replacement with fibrous tissue, which may be a sequel to primary or secondary hyperparathyroidism. This report intends to document the clinicopathological findings of ODF in a flock of young goats fed primarily on a wheat bran-rich diet. **Case description:** In a flock of 50 stall-fed goats aged 1 to 2 years, seven were clinically presented with bilateral facial enlargement, leading to dyspnea and difficulty in prehension and mastication. Among the seven clinically affected goats, four died in 2 months. **Findings/treatment and outcome:** The clinical examination revealed bilateral mandibular enlargement and limb deformities. On radiography, the maxilla and mandible had decreased radiopacity. Fine needle aspiration cytology (FNAC) from the affected bones showed occasional fibroblasts and individual osteoclasts clusters. On necropsy, the enlarged mandible revealed a meaty consistency. Undecalcified histological sections of the mandible showed severe osteopenia, multiple osteoclasts, Howship's lacunae, and extensive fibroplasia. Dietary corrective measures led to the prevention of ODF in the rest of the flock. **Conclusion:** Excessive wheat bran feeding in stallfed goats might have led to calcium and phosphorus imbalance, resulting in nutritional secondary hyperparathyroidism and subsequent skeletal deformities. FNAC of the affected bones, gross and histological findings provide a clinicopathological diagnosis of ODF.

Key words: Goat, Osteodystrophia fibrosa, Secondary hyperparathyroidism

Introduction

Osteodystrophia fibrosa (ODF) is a metabolic skeletal disorder attributable to the prolonged and widespread action of parathormone (PTH) on the skeletal system. It is caused either by primary hyperparathyroidism, due to a lesion in the parathyroid gland itself, or secondary hyperparathyroidism, induced by hyperphosphatemia resulting from nutritional imbalance or renal impairment (Thompson, 2007). In domestic animals, hyperparathyroidism-associated bone malformations often are named as ODF, fibrous osteodystrophy, bran disease, rubber jaw syndrome, big head disease, etc (Bharti *et al*., 2021). ODF is characterized by severe resorption of calcified bone matrix, inadequate mineralization of immature osteoid, and replacement of bone trabeculae with proliferating connective tissue (Ozmen *et al*., 2017). In the majority of domestic animals, nutritional imbalance and subsequent lowered serum calcium levels, as well as elevated phosphorus levels, result in increased synthesis and secretion of PTH, which plays the key role in the pathogenesis of ODF by activation of osteoclast-mediated demineralization of bones (Thompson, 2007). Although it is a generalized bone disorder, the maxilla and mandible are more prone to osteodystrophy due to their higher renovation rate and in response to enormous and repeated mechanical stress during the mastication (Doige and Weisbroge, 1995).

The species susceptibility to ODF varies among different domestic animals; nutritional ODF is documented frequently in equines (Radostits *et al*., 2008; Stewart *et al*., 2010), sporadically in goats (Aslani *et al*., 2001; Pavarini *et al*., 2011), camels (Lynch *et al*., 1999), and rabbits (Bas *et al*., 2005). Among ruminants, the incidence of ODF is relatively higher in goats and rare in cattle and sheep (Pavarini *et al*., 2011). In goats, all age groups are susceptible with no breed or sex predispositions documented (Stewart, 2010; Smith and Sherman, 2011). Young animals raised on diets containing low calcium and high phosphorus are mostly affected by ODF (Radostits *et al*., 2007). Enlargement of the facial bones, especially the mandible and maxilla, difficulty in closing the mouth, and tongue protrusion with abnormalities in prehension and mastication are the characteristic clinical indications of ODF (Varshney *et al*., 2018). Bending of the lower extremities of limbs and enlargement of joints may also occur in some cases of ODF (Aslani *et al*., 2001; Ozmen *et al*., 2017). The occurrence of ODF and its methods of clinical diagnosis were not well documented in goats. Clinical signs, radiography, and histopathology of affected bones are used to diagnose in most cases. The diagnostic application and reliability of the fine needle aspiration cytology (FNAC) technique in caprine ODF still need to be documented in any case. In light of this, the objectives of the current study are to document the characteristic gross, histopathological, and radiographic findings of ODF and to investigate the application of FNAC as a reliable method for the clinical diagnosis of ODF in goats.

Case description

Seven goats were clinically presented with a history of spontaneously developing progressive and bilaterally symmetrical enlargement of the facial region since 5 to 8 weeks from a flock of 50 Rohikhandi goats (an indigenous goat breed of India) of either sex, aged approximately 1 to 2 years, reared in an intensive system, and fed exclusively on a concentrate mixture consisting of approximately 60% wheat bran and 40% maize for the previous 8 months. On clinical examination, all seven goats had poor body condition, pallor of visible mucous membranes, varying degrees of symmetrical enlargement of the mandible predominantly towards the angle, tongue protrusion, dyspnoea, difficulty in prehension and mastication, and varying degrees of bilateral exophthalmos (Fig. 1a). Four of the goats also had forelimb conformational defects, such as forward bending of the knee joints and enlargement and lateral deviation of the elbow joints (Fig. 1b), which resulted in marked limping while walking. Four goats died within two months of their clinical presentation. From the clinical findings and the nutritional history, the condition was tentatively diagnosed as osteodystrophia fibrosa, and further investigations were conducted to confirm the diagnosis.

Findings/treatment and outcome

Hematology

Blood samples were collected from all seven clinically affected goats. On hematological evaluation, all goats showed significant reduction in haemoglobin content and haematocrit without any overt reticulocytosis, indicating marked nonregenerative anemia.

Diagnostic imaging

Radiography of the skull of the clinically presented goats revealed diminished radio-opacity of the mandible and maxilla in a diffuse pattern, marked enlargement of the mandible predominantly towards the angle of the mandible, and a unilocular expansile radiolucency at the mandibular symphysis (Fig. 2).

Fig. 1: Clinical findings of Osteodystrophia fibrosa. (**a**) Bilaterally symmetrical enlargement of the facial region and marked exophthalmia, and (**b**) Abduction of the elbow joint and bending of the carpo-metacarpal region of forelimbs (arrow)

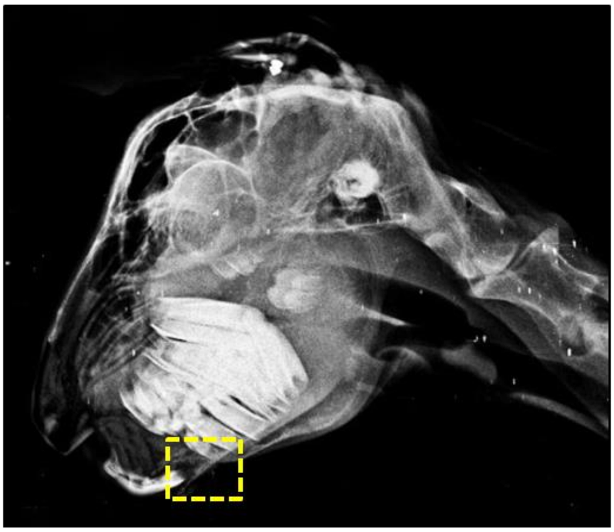

Fig. 2: Left lateral radiograph of the head of a goat with Osteodystrophia fibrosa. It shows diffusely decreased radio-opacity of the mandible and maxilla, marked enlargement of the mandible predominantly towards the angle of the mandible and a unilocular expansile radiolucency at the mandibular symphysis (square)

Fine needle aspiration cytology (FNAC)

FNAC smears from various sites of the enlarged mandible of the goats showed abundant proteinaceous background material, occasional clusters of fibroblasts, few aggregates of binucleated chondrocytes, and individual multinucleated osteoclasts, indicating osteopenia with fibrous tissue proliferation (Figs. 3a-c).

Necropsy findings

Out of seven clinically ill goats, four goats that presented with respiratory distress later died and were presented for necropsy examination. The most consistent and significant finding in all goats was a marked bilateral and symmetrical enlargement of the mandible, most notably towards the angle of the mandible with a raised irregular contour, and the entire bone was soft and flexible on palpation. All the teeth on the lower jaw were misaligned and shaky, and some were exfoliated (Figs. 4a-f). The mandible was easy to cut with a knife and had a meaty consistency as opposed to the hard and gritty consistency of the bone. The cut surface was pale and firm. The palatine process of the maxilla and nasal bones was also flexible and easy to incise. All goats had poor nutritional status with reduced muscle mass and marked depletion of subcutaneous, mesenteric and omental fat depots, as well as severe serous atrophy of epicardial adipose tissue, indicating inanition due to jaw abnormalities.

Histopathology

The bone and parathyroid samples were fixed in 10% neutral buffered formalin, and after routine tissue processing, the tissue sections were stained with haematoxylin and eosin (Luna, 1968). The undecalcified sections of the affected mandible showed extensively proliferating fibrous connective tissue replacing the calcified bone matrix. The calcified bone matrix and

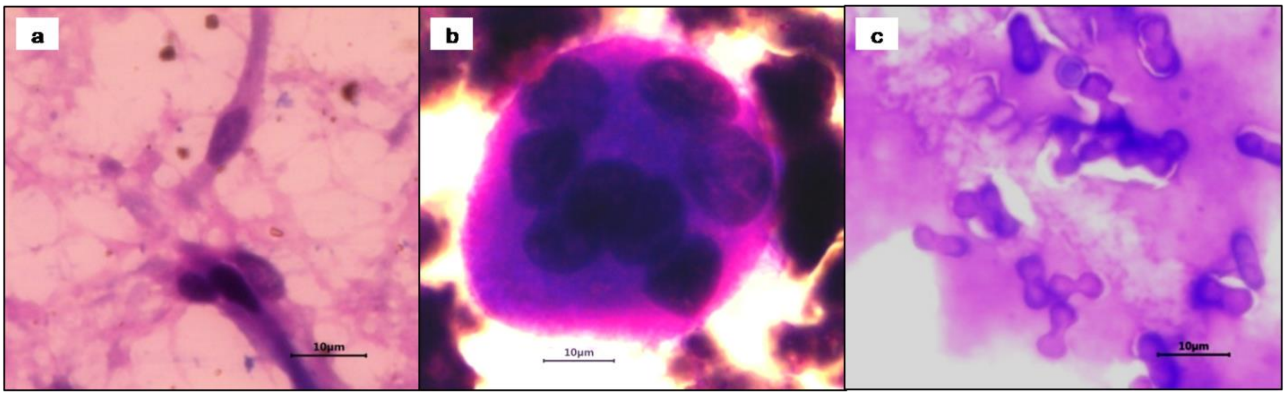

Fig. 3: Microscopic evaluation of fine needle aspiration cytology of the mandible (Giemsa stain, ×1000). Scanty cellular smears with abundant proteinaceous background material, (**a**) Clusters of fibroblasts, (**b**) Individual multinucleated osteoclast, and (**c**) Aggregates of binucleated chondrocytes

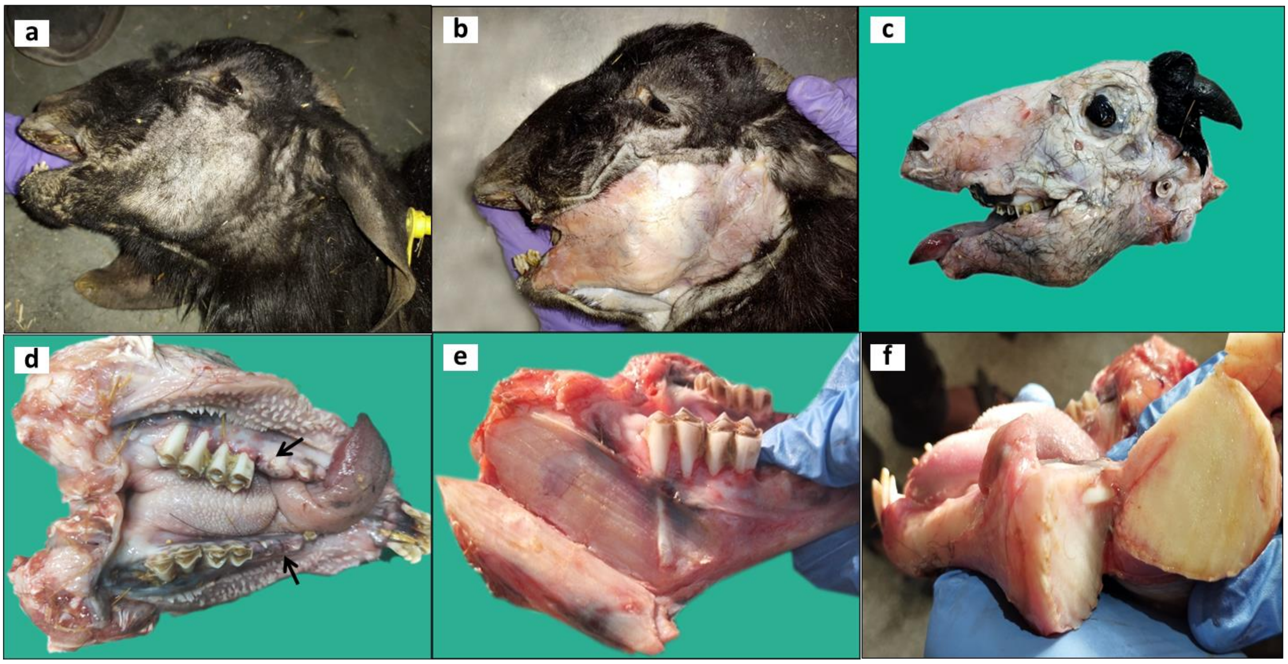

Fig. 4: Necropsy findings. Photographs of (**a**) left lateral view of head after trimming hairs over the facial region showing marked enlargement of the lower jaw with a raised irregular contour and widely opened mouth, which was unable to close even by applying pressure, (**b**) After skinning the facial region, showing the enlargement more apparent towards the angle of mandible, (**c**) After removal of the muscles and fasciae over the face revealing the exclusive involvement of bone in the facial swelling, (**d**) Lower jaw showing marked bilateral enlargement of mandible, misaligned and shaky teeth, moderately congested gingival margins of molar teeth and absence of all sets of premolars (arrows), (**e**) Sagittal section of right half of enlarged mandible, and (**f**) transverse section of left half of enlarged mandible at the angle showing uniform pale pink flesh like cut surface

immature osteoid were distributed sparsely among the proliferating fibrous tissue (Figs. 5a and b). The remnants of calcified bone trabeculae showed irregular grooves of bone resorption (Howship's lacunae) containing large multinucleated osteoclasts with ruffled cell borders. Sections from the maxilla, radius, and metacarpals also showed osteopenia and fibrosis, albeit to a lesser extent. The fibroproliferative lesions of bone were also progressively invading marrow spaces and impinging on the haematopoetic cells. The parathyroid gland showed moderate degrees of hyperplasia and hypertrophy of the chief cells (Fig. 5c). The degree of demineralization and fibrosis were assessed by special staining methods, Von Kossa silver nitrate staining (Fig. 6a) and Masson's trichrome staining (Fig. 6b), respectively, as previous described protocol (Sharma *et al*., 2022).

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections of the affected mandible were deparaffinized and hydrated. Endogenous peroxidase activity was blocked by incubating in 3% H_2O_2 for 20 min. Nonspecific staining was blocked with 5% rabbit serum for 30 min. Then the sections were incubated with polyclonal anti-proliferating cell nuclear antigen (PCNA) primary antibody (Catalog No. AV03018, Sigma, USA), at a dilution of 1:100, overnight at 4°C. Then the tissue sections were sequentially incubated with goat anti-rabbit IgG secondary antibody (Catalog N. 31460; Sigma) at a dilution of 1:200 for 1 h at 37°C. Diaminobenzidine (DAB; Sigma) was used as a chromogenic substrate and then counterstaining was done with hematoxylin. In these sections, the fibroblasts showed strong nuclear immunoreactivity, indicating an ongoing active fibroplasia (Fig. 6c).

Treatment and follow up

Out of the seven animals, four succumbed to death and the remaining three were culled. The owner was advised to replace bran feeding with a balanced feed and appropriate calcium and vitamin D supplementation for the remaining flock. Over an observation period of 6 months, no new cases of ODF were reported from the flock.

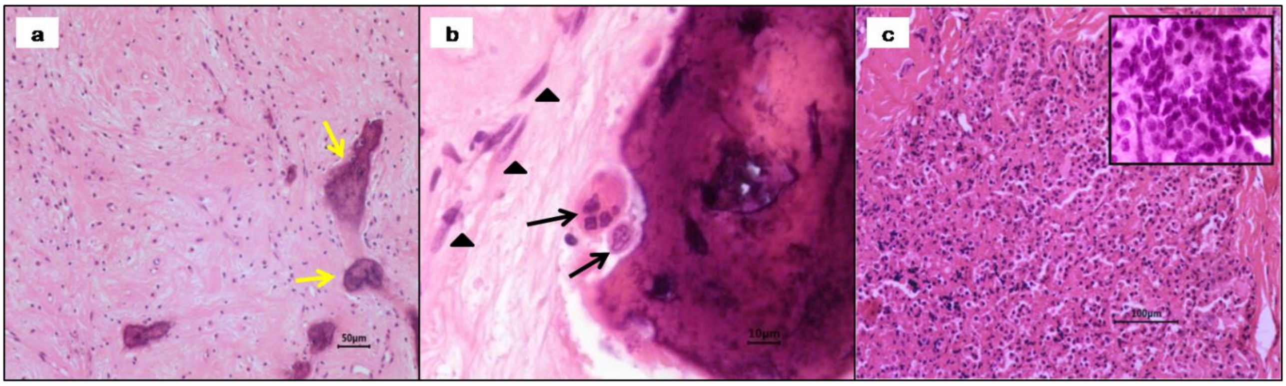

Fig. 5: Histopathological findings. (**a**) Undecalcified section of the mandible showing extensively proliferating fibrous connective tissue and sparsely distributed remnants of calcified bone trabeculae and immature osteoid (yellow arrows) [H&E, ×40], (**b**) Undecalcified section of the mandible showing remnants of calcified bone matrix with irregular grooves of bone resorption (Howship's lacunae) containing large multinucleated osteoclasts with ruffled cell borders (black arrows), the surrounding areas consist of bundles of fibroblasts (arrow head) and fibrous tissue deposition [H&E, ×400], and (**c**) Parathyroid gland showing hyperplastic chief cells with crowding, enlargement and impinging the fibrovascular stroma [H&E, ×100] (Inset-Showing closely packed chief cells with hyperchromatic nuclei [H&E, ×400])

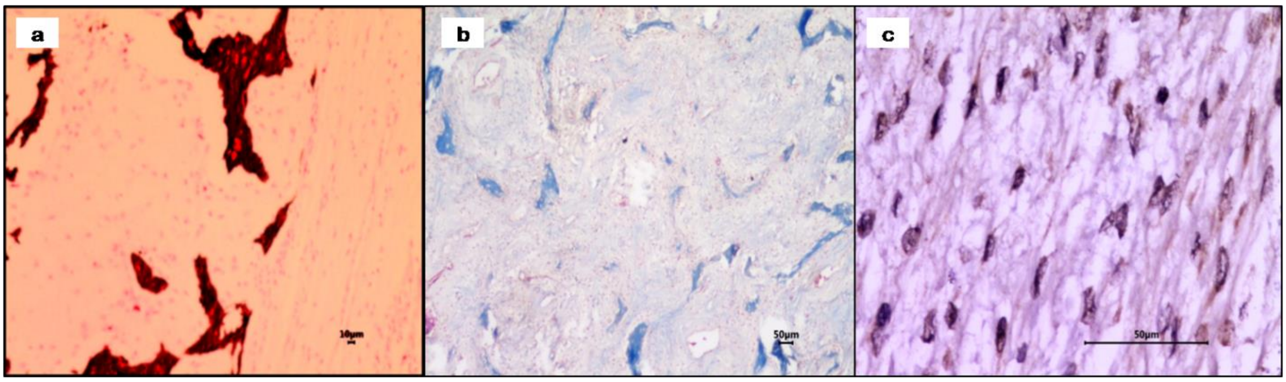

Fig. 6: Undecalcified sections of the mandible. (**a**) Von Kossa stain demonstrating sparsely distributed black stained calcified bone trabeculae (von Kossa stain, ×100), (**b**) Masson's trichrome stain showing extensive deposition of collagen fibers (blue) of various levels of maturation and immature osteoid (Masson's trichrome stain, ×40), and (**c**) Fibroblasts showing strong nuclear PCNA immunostaining (DAB, ×400)

Discussion

Nutritional osteodystrophy arises as a metabolic bone disorder in animals subjected to prolonged consumption of a diet characterized by low calcium and elevated phosphorus content. This dietary pattern leads to hypocalcemia and hyperphosphatemia, causing sustained upsurges of PTH levels. PTH stimulates osteoclast-driven bone resorption, replacing the resorbed bone with fibrous connective tissue (Aslani *et al*., 2001; Ozmen *et al*., 2017). In the current study, goats were exclusively fed a concentrate diet abundant in wheat bran for 8 months, devoid of green feeding. Wheat bran, known for its elevated phosphorus content, exhibits a Ca:P ratio of approximately 1:12.6 (Heuze *et al*., 2015). The provided diet demonstrated a substantial imbalance between calcium and phosphorus, displaying a markedly reduced Ca:P ratio, significantly divergent from the recommended ratio of 2:1 (Radostits *et al*., 2007). Caprine nutritional ODF exhibits a higher incidence among animals raised in confinement than those engaged in grazing, a correlation noted in the current study and supported by prior research (Pavarini *et al*., 2011). The dietary imbalance of minerals and concurrent vitamin D deficiency resulting from limited sun exposure within the stallfeeding system plausibly precipitated the early manifestation of ODF. In this study, the affected animals, aged 1-2 years, represent a notably susceptible age group characterized by high rates of bone turnover and remodeling, as evidenced by Thompson (2007).

In the present study, the condition was diagnosed as nutritional ODF based on nutritional history and clinical and pathological findings. Although it is a generalized skeletal disorder, the enlargements of the facial bones are the most characteristic and consistent lesion in caprine nutritional ODF (Thompson, 2007; Varshney *et al*., 2018); less frequently, the involvement of other regions like the appendicular skeleton is also documented in some cases (Aslani *et al*., 2001; Ozmen *et al*., 2017). All the affected animals were severely emaciated and anemic, which might be due to the inanition as a result of difficulty in prehension and mastication, and four animals showed dyspnoea and open mouth breathing, which might be due to the narrowing of the upper respiratory tract resulting from the enlargement and distortion of the maxilla and mandible. Findings of radiographic examinations of the affected goats' skulls concurs with the findings of Varshney *et al*. (2018). The application of FNAC in the clinical diagnosis of ODF has not yet unraveled, and no data is available on the cytopathological findings of ODF; the current study proves the usefulness of FNAC in the diagnosis of ODF since it gives clear indications of increased activity of osteoclasts along with fibroplasia.

Histopathology would be the most powerful confirmatory diagnostic tool in ODF if a biopsied or morbid sample of the affected bone is available. The characteristic histopathological pictures of the ongoing process of bone resorption, showing an increased number of Howship's lacunae containing multiple osteoclasts and replacement of the demineralized bony matrix with proliferating fibrous tissue, were obtained in this study as documented in previous studies (Aslani *et al*., 2001; Thompson, 2007; Pavarini *et al*., 2011). In the present study, active fibroplasia in the bone was demonstrated by immunolocalization of PCNA in fibroblasts. PCNA is a marker of cellular proliferation mainly synthesized in the G1 and S phases of the cell division cycle (Strzalka and Ziemienowicz, 2011).

The histopathological examination of the parathyroid gland of affected goats showed moderate hyperplasia and hypertrophy of the chief cells, indicating long-standing stimulation of the gland and progression of secondary hyperparathyroidism to tertiary, which might have led to autonomous overproduction of PTH as well. There is only limited knowledge about the treatment of the disease. The outcome of therapeutic management of caprine nutritional ODF varies with the severity of the clinical signs. In the case of an early diagnosis of the condition, corrective dietary measures involving a reduction of phosphorus intake and balancing the calcium-phosphorus ratio will help reverse clinical lesions (Akter *et al*., 2018). In the present study, out of the seven animals, four succumbed to death, and the remaining three were culled, after following the corrective measures such as discontinuation of bran feeding, providing balanced feed with calcium and vitamin D supplementation, and allowing access to direct sunlight, led to the cessation of ODF in the rest of the flock.

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Conflict of interest

Authors have no conflict of interest to declare.

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