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Research Article

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Comparative Histology and Micrometric Analysis of Pharyngeal Cavity of Egyptian Laughing Dove (*Streptopelia senegalensis aegyptiaca*) and Japanese Quail (*Coturnix coturnix japonicum*)

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Abstract

Birds of different families; Japanese Quail (family Phasianidae) and Laughing Dove (family Columbidae) were used for this study to focus on the histological and micrometric comparison of the pharyngeal roof and floor with its content. The thickness of the lining epithelium of the pharyngeal roof, root of the tongue was almost equal in both species, whereas of the laryngeal mound of quail was nearly 5 times thicker than that of dove. On the other hand, the diameter of the glandular lobules of all studied salivary glands in the pharyngeal cavity of quail was more than that of dove except caudal lingual salivary glands. The entrance of the infundibular cavity of dove was infiltrated by aggregations of lymphoid tissue, but there were variably sized lymph nodules in quail termed pharyngeal nodules. The sphenopterygoid salivary glands (branched tubular mucous type) of dove were fewer than those of quail. The caudal lingual salivary glands were concentrated centrally dorsal to basihyoid of the hyoid bone in dove but distributed dorsolaterally to the basihyoid in quail. The lamina propria of the laryngeal mound of dove had 2 groups of circopterygoid salivary glands (rostromedial and caudomedial), lining by tall columnar epithelium, while of quail had 3 groups (rostromedial, rostrolateral and caudomedial) circopterygoid salivary glands, lining by low columnar epithelium. The laryngeal mound of both species was supported by two groups of intrinsic laryngeal muscles.

Keywords: Dove, Infundibular slit, Laryngeal mound, Lingual root, Quail, Salivary glands.

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coturnix japonicum). SVU-IJVS 2020, 3(2): 115-129.

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INTRODUCTION

The Egyptian laughing dove is a bird dwells farmland and feeding on seeds, grasses and vegetable material (Satheesan et al., 1990, Adang et al., 2008), these species are found in the Nile valley (Peters, 1937). The Japanese quail is one of the species inhabits the ground and depends on shrubs, agricultural fields such as oats, rice, and barley for nutrition (Buchwalder and Wechsler, 1997, Pappas, 2013). The nutrition, food intake, and ingestion of the birds are affected by the structure variations of the digestive system especially oropharynx (Jayachitra et al., 2015). The pharynx participates tongue and jaw during various behaviors such as feeding and drinking (Homberger and Meyers, 1989). glandular tissue is the most The characteristic feature of the oropharynx of avian and was explained in several species of birds (Jackowiak and Godynicki, 2005, Almansour and Jarrar, 2007, Dehkordi et al., 2010, Igwebuike and Eze, 2010, Pasand et al., 2010, Crole and Soley, 2011). Salivary glands are well developed in seed or insect-eater birds (dry food eating birds), least developed in fish-eater birds (welllubricated diet eating birds), and totally absent in a few species as the Great Cormorant (King and McLelland, 1984). The salivary glands frequently secret mucus but may also secret some amylase (Orosz, 1997). In addition to the basic function of the saliva i.e. humidification and lubrication of food, it forms a barrier between the oral mucosa and the foreign materials as bacteria, mechanical damage, toxin (Samar et al., 2002, Crole and Soley, 2011), some birds produce salivary secretions for other uses as sticky secretions to catch insects, glue for attaching nests to the wall, to build nests, to form boluses for winter food supply, and others (Orosz, 1997).

Several studies have mainly focused on the anatomical and scanning electron investigations microscopical of the oropharynx in different species of birds (Tajali et al., 2008, Igwebuike and Eze, 2010, Erdogan and Alan, 2012, Moussa and Hassan, 2013, Erdoğan and Pérez, 2015, Jayachitra et al., 2015, Abumandour and El-2017a, Abumandour, Bakary, 2018, Mahdy, 2020). While, the histological studies and micrometric analysis, especially on the pharyngeal cavity seems to be less sufficient, so this study supported a sufficient data on the pharyngeal cavity of species fly (dove), not fly (quail) which have different feeding habits and habitats with corresponding differences in the structures of their oropharynx to adapt to their different environment.

MATERIALS AND METHODS

Eight adult birds were used for this study; four doves (94.26 ±8.02gm) were procured from bird hunters and four quail $(235.67\pm2.5\text{gm})$ were collected from the researcher's farm in South Valley University, Qena governorate, Egypt. After scientific sacrifice, the heads were dissected and washed with tap water and saline. Then the pharyngeal roofs and floors were dissected from the head of the birds. The root of the tongue and laryngeal mound were cut in cross sections, laryngeal mound was cut in 2 parts; cranial and caudal at its caudal commissure. The sacrifice of the birds followed the Institutional Ethical Committee in Faculty of Veterinary Medicine, SVU. The samples were washed, fixed in 10% neutral buffered formalin, then dehydrated in ascending grades of alcohol (70% for overnight, 80% for 2h, 90% for 2h, 100% for 1h). After dehydration, the samples were transferred to methyl benzoate 24h for clearance then embedded in paraffin wax (PI, PII, PIII) 3h for each one except the last for overnight. Serial

sections (3-5µm thickness) were cut using (LEICA 2165) microtome and mounted on glass slides. The sections were subjected to a descending series of alcohol after good dewaxing in xylene, the previous technique according to Bancroft et al. (2013). Finally, the slides staining by Harris hematoxylin and eosin stain (Harris, 1900), Periodic-Acid Schiff stain (McManus, 1946). The examination of the stained sections by using Leitz Dialux 20 Microscope and a Canon digital camera (Canon Powershot A95) was used for taken photos. The different measurements were taken in this study by using Image J software after that analyzed by the (SPSS) software program, version 17.0 (Argyrous, 2011).

RESULTS

The pharyngeal cavity was represented the caudal part of the oropharynx. It extended from the caudal end of the choanal slit dorsally and a transverse row of the caudally directed lingual papillae ventrally to the pharyngoesophageal junction. The wall of the pharynx in both species was consisted of mucosa, thin layer submucosa, muscular layer (pharyngeal muscles).

Pharyngeal roof

The mucosa of the pharyngeal roof was consisted of lamina epithelialis and lamina propria. The lamina epithelialis was formed of stratified squamous noncornified epithelium except epithelial parakeratinization was apparent near the edges of the infundibular slit, especially on the pharyngeal papillae which were mostly conical in quail. The thickness of the epithelial layer of the pharyngeal roof near infundibular slit was 302.48± 8.29 µm in dove and 318.95±22.80 µm in quail. This epithelium was transformed into ciliated pseudostratified columnar epithelium (respiratory epithelium) near the inlet of the

infundibular slit in dove, but away from it by a distance in quail (Fig.1). The respiratory epithelium was interrupted by intraepithelial glands, these glands were the simple and mucous type connected to short ducts open in the infundibular cavity, easily identifiable in dove while in quail these glands were completely obliterated and covered by dense aggregations of lymphoid tissue. The secretory lining cells of the intraepithelial glands were columnar with basally located oval nuclei, and the cytoplasm was foamy and vacuolated (Fig.1). The lamina propria was occupied by groups of branched tubular mucous secretory glands known as sphenopterygoid salivary glands, which were fewer in dove than in quail, concentrated mainly on both sides of the infundibular slit extended into the wall of the infundibular cavity in quail (Figs.1, 2).



Fig. (1): Photomicrographs of the cross section of the pharyngeal roof of laughing dove (A, B), of Japanese quail (C, D), showing non-cornified stratified squamous epithelium (LE) except at pharyngeal transformed papillae (P) into ciliated pseudostratified columnar epithelium (arrowheads) interrupted by intraepithelial mucous glands (g) connected with duct (arrow) open into infundibular cavity (IFC), surrounded and obliterated by lymphatic infiltration (LI) and lymphatic nodules (LP) (LN), lamina propria contained sphenopterygoid salivary glands (ssg), pharyngeal muscle (pm). H&E stain.

The lining epithelium of the secretory units of each lobule was low columnar in dove and tall columnar in quail. The secretory cells with basally located flat nuclei with foamy, highly vacuolated cytoplasm (Fig.2).



Fig. (2): Photomicrographs of the sphenopterygoid salivary glands of laughing dove (A), of Japanese quail (B-D), showing lining epithelium of the secretory units of the glands (ssg) was low columnar (black arrow) and tall columnar (red arrow), duct epithelia of these glands changed from stratified squamous type (arrowhead) to low columnar type (double arrowheads). H&E stain.

These cells showed stronger positive reactions for PAS in quail than that of the dove. And also, those of the intraepithelial mucous glands showed intense PAS reaction (Fig.3). The mean diameter of the lobules of the sphenopterygoid salivary glands was $518.29\pm7.04 \ \mu m$ in dove and $562\pm11.49 \ \mu m$ in quail.

The ducts of the sphenopterygoid salivary glands passed through (crossed) the stratified squamous epithelium of the pharynx and thus ducts openings were lined for a short distance with this epithelium which changed gradually to low columnar cells extending down to the common cavity of the gland, where it increased in height to become the typical salivary secretory epithelium (Fig.2).



Fig. (3): Photomicrographs of the cross section of the pharyngeal roof of laughing dove (A), of Japanese quail (B), showing positive PAS reactions for lining epithelium of sphenopterygoid salivary glands (ssg) and of intraepithelial mucous glands (g). PAS stain.

In both species, the lamina propria toward the infundibular cavity was infiltrated with aggregations of lymphoid tissue, and surrounded by an ill-defined connective tissue capsule in dove, furthermore, the lymphoid tissue overlying the epithelium and associated with the intraepithelial glands especially at the entrance of the infundibular cavity. Unlike in quail this lymphoid tissue was completely overlying the epithelium and forming variably sized aggregations of lymph nodules which represented the pharyngeal lymph nodules. The pharyngeal

muscles of the dove were thicker than that of quail (Fig.1).

Pharyngeal floor

The stratified squamous epithelium of the pharyngeal floor was lined the lingual root and continuous caudally with the epithelium of the laryngeal mound till the edges of the glottis.

The lamina epithelialis of dorsum of the lingual root was non-cornfield except epithelial parakeratinization was observed on the rounded-shaped horny papillae in quail and large conical-shaped papillae in dove which had the transverse row of the caudally directed lingual papillae. These papillae were consisted of connective tissue core infiltrated with lymphocytes and associated with caudal lingual salivary glands and plate of ceratobrachialis cartilage of the hyoid bone in dove. The stratified epithelium of the lingual root of the dove was continued by the same thickness toward the floor and lateral wall of the pharynx, whereas of quail was markedly increased. The thickness of the lingual epithelium was $188\pm11.55 \ \mu m$ in dove and $212.53\pm2.80 \ \mu m$ in quail.

The lamina propria of the lingual root mucosa was formed of dense connective tissue contained a group of caudal lingual salivary glands concentrated centrally, dorsal to basihyoid of the hyoid bone in dove but distributed dorsolaterally to the basihyoid in quail. Moreover, in dove, abundant aggregation of lymphocytes within lamina propria was associated with the lingual salivary glands which markedly increased toward the pharyngeal wall and within the caudal mandibular salivary glands (Fig.4).



Fig. (4): Photomicrographs of the cross section of the lingual root of laughing dove (A-C), of Japanese quail (D, E). (A, D) Non-cornified stratified squamous epithelium (LE) except at papillae of the transverse row (P), lamina propria (LP) contained caudal lingual salivary glands (Lsg) located above central cartilaginous plate of the basihyoid bone (BH) which surrounded by masses of intrinsic lingual muscle (IM), caudal mandibular salivary glands (msg) were demonstrated at the floor of the pharynx (ph). (B) Papilla with connective tissue core infiltrated with aggregations of lymphocytes (LI) associated with caudal lingual salivary glands (Lsg) and plate of ceratobrachialis cartilage (cb) of the hyoid bone. (C, E) Secretory units of caudal lingual salivary glands (Lsg) lining by columnar epithelium.

The secretory units of these glands of dove were lined by tall columnar cells with flat basally located nuclei and foamy, vacuolated, and faintly stained cytoplasm, but they were low columnar cells with deeply stained basophilic cytoplasm in quail. The mean diameter of the glandular lobules of the caudal lingual salivary glands was $497.34\pm3.47\mu$ m in dove and $353.39\pm35.63\mu$ m in quail. The lingual core of the root consisted of a central cartilaginous plate of the basihyoid bone which became ossified and surrounded by bulk of intrinsic lingual muscles (Fig.4).



Fig. (5): Photomicrographs of the cross section of the rostral part of the laryngeal mound of laughing dove (A-C), of Japanese quail (D-F). (A, D) stratified squamous epithelium (arrow) transformed into respiratory system (arrowhead), lamina propria (LP) contained rostromedial cricoarytenoid glands (1csg), rostrolateral cricoarytenoid glands (*1csg), pharyngeal glands (Pg), laryngeal cavity (LC), Laryngeal inlet (LI) supported by paired arytenoid cartilages [body (**AC), rostral process (*AC)], cricoid cartilage (CC). These cartilages were connected by intrinsic laryngeal muscles (IM) and surrounding externally by extrinsic laryngeal muscles (EM). (B, E) Ciliated pseudocolumnar epithelium (double arrowheads) interrupted by intraepithelial mucus glands (g). (C) Glandular lobules of rostromedial cricoarytenoid glands (1csg) lining by high columnar epithelium and surrounded by thick connective tissue capsule (CT), intrinsic muscles (IM). (F) Cricoarytenoid glands (csg) with its epithelium low columnar (arrow), its duct epithelium was converted gradually from cuboidal type (barbed arrow) to stratified squamous epithelium (LE).

The laryngeal mound was lined non-cornified stratified externally by squamous epithelium till the edges of the laryngeal inlet which represented the continuation of the lining epithelium of the pharynx then transformed into ciliated pseudostratified columnar epithelium (respiratory epithelium) near the laryngeal inlet in dove but by a distance in quail (Fig.5). The keratinized epithelium was demonstrated only at the median of the caudal part of the laryngeal mound toward its caudal commissure in quail. The epithelium of the latter area of dove was interrupted by intraepithelial mucous secretory glands, opened into the pharyngeal cavity (Fig.6). The thickness of the epithelial layer lining the laryngeal mound near the laryngeal slit was measured 63.58±6.20 µm in dove and 363.2±21.67 µm in quail. The ciliated respiratory epithelium was more developed in dove than quail, contained intraepithelial mucous glands which was lined by a single layer of low columnar mucous secreting cells with small flattened, basally located nuclei and foamy, vacuolated cytoplasm (Fig.5).

The lamina propria of the rostral part of the laryngeal mound of dove had one group of salivary glands (rostromedial cricoarytenoid glands), whereas two groups (rostromedial, rostrolateral cricoarytenoid glands) could be detected in quail on both sides of the rostral part of the laryngeal inlet. These glands were numerous in quail, continued laterally till the lateral wall of the pharynx and appearance the pharyngeal salivary glands. Whereas the caudal part of the laryngeal mound has one group in both birds (caudomedial cricoarytenoid glands) were demonstrated on both sides of the caudal commissure of the larvngeal mound (Figs.5,6). The glandular lobules of these glands of dove were surrounded by thicker connective tissue capsule and the secretory units were lined by tall columnar

epithelium. Whereas of quail, the lining epithelium of the glands was low columnar epithelium (Fig.5). The mean diameter of the glandular lobules of cricoarytenoid glands was 245.51±29.81 µm in dove and 284.25±16.92µm in quail. The ductal epithelia of those glands were converted to cuboidal type and gradually toward the pharyngeal surface became stratified squamous epithelium which considered as the continuation of the surface epithelium mucosa. of pharyngeal The the cricoarytenoid salivary glands were mucous type showed intensive PAS reaction. The ducts of some glands were observed penetrating the mucosa to open in the pharyngeal cavity (Figs.5, 6).



Fig. (6): Photomicrographs of the cross section of the caudal part of the laryngeal mound at the caudal commissure of laughing dove (A, B), of Japanese quail (C, D), showing stratified squamous epithelium (LE) interrupted by intraepithelial mucous glands (g) and keratinized at median part (arrow) toward caudal commissure, laryngeal sulcus (LS), lamina propria (LP) contained caudomedial cricoarytenoid glands (2csg), laryngeal cavity (LC) supported by paired arytenoid cartilages [body (**AC), caudal process (*AC⁺)], procricoid cartilage (PC), cricoid cartilage (CC), intrinsic laryngeal muscles (IM), extrinsic laryngeal muscles (EM).

The laryngeal inlet was supported dorsally by paired arytenoid cartilages and caudodorsally by procricoid cartilage and ventrolaterally by cricoid cartilage. The ossification was detected in the body of arytenoid, but its processes remain cartilaginous as well as procricoid of quail was ossified formed of trabecular bone. These cartilages were connected by intrinsic laryngeal muscles arranged in two groups and surrounding externally by three groups of extrinsic laryngeal muscles, these muscles of quail were markedly thicker than that of dove (Figs.5,6).

DISCUSSION

The present study clarified that the surface epithelium of the pharynx was stratified squamous type non-cornified in both species except epithelial parakeratinization was apparent near the edges of the infundibular slit especially on the conical papillae. Hodges (1974) mentioned that the pharynx in fowl is lined by a stratified squamous epithelium, lacking in a surface cornified layer. The rostral pigmented part of the oropharynx of emu is keratinized while caudal nonpigmented part is non-keratinized (Crole, 2011). In turkey, the surface epithelium of the pharyngeal roof is cornified rostrally and non-cornified caudally with the thick epithelium (Sayed et al., 2016). Our results confirmed the reports mentioned by King and McLelland (1984) that the cornification and non-cornification of the epithelium in birds depending on their feeding habits. The keratinization of the oropharyngeal epithelium occurs in varying degrees (Nickel et al., 1977, McLelland, 1979, King and McLelland, 1984) and the degree of keratinization differs according to the degree of the exposure to mechanical stress caused by food ingredients (Nickel et al., 1977). The current study supported the results of Madkour (2018) that the surface of the pharyngeal roof of the dove was free from papillae but characterized by conical papillae of different sizes in quail. The observation recorded in the dove was

similar to the observation reported in young pigeons (Mahdy, 2020), and the histoarchitecture observed in quail was tallied with turkey (Sayed et al., 2016), hooded crow (Moussa and Hassan, 2013). The pharyngeal papillae play an important role in transporting bolus toward esophagus (König et al., 2016).

In agreement with the previous data indicated that the lymphatic aggregations were surrounded the intraepithelial mucous glands near the edges of the infundibular slit (Hassouna, 2002, Sayed et al., 2016, Madkour et al., 2020). Because of quail was terrestrial birds, eating with rapacity, exposure to inhale, and ingest foreign bodies than dove which was flying birds, our study elucidated in quail that the lymphoid tissue forming variably sized pharyngeal lymph nodules obliterated the glands corresponding with the reports mentioned by Crole and Soley (2012) in Dromaius novaehollandiae and Struthio camelus, Crole (2011) in emu, Tivane (2008) in ostrich. The oropharynx of the birds characterized by abundant lymphoid tissue (Rose, 1981), mainly situated in the pharyngeal cavity (Barge and Mundhöhlendach, 1937, McLelland, 1979). This lymphoid tissue is termed pharyngeal tonsil (Rose, 1981, Berens von Rautenfeld, 1993, Casteleyn et al., 2010). The lymphoid tissue concentrated as tonsils throughout other different parts of the digestive system of birds as esophageal, pyloric, and cecal tonsils (Casteleyn et al., 2010). On the other hand, the current finding had demonstrated lymphocytes aggregation of at the demarcation between oral and pharyngeal floors which was associated with the caudal lingual salivary gland and within caudal mandibular salivary glands of dove.

The histological results showed that all salivary glands lying in the pharynx of both species were observed in the lamina propria. Several published articles were boosted these results (salivary glands in pharynx located within lamina propria) as lingual glands in duck (Mohamed, 2019), spheniopterygoid glands in turkey (Sayed et al., 2016) and duck (Madkour et al., 2020), as well as, Hodges (1974) stated that the glands in pharynx at base of lamina propria or within submucosa. Whilst large number of data are demonstrated salivary glands within submucosa in different avian species as caudal lingual glands in laughing dove (Abumandour and El-Bakary, 2019), quail (Uppal et al., 2014), kestrel and owl (Abumandour and El-Bakary, 2017b), penguin (Kobayashi et al., 1998) and circoartynoid salivary glands in duck (Mohamed et al., 2018).

In the same line with several researchers identified that the most common type of gland in birds is tubular type (Chodnik, 1948, Calhoun, 1954, Banks, 1993, Samuelson, 2007, Sağsöz et al., 2013). While the other identified as simple branched tubuloalveolar, alveolar, and complex alveolar glandular structures in birds (Samar et al., 1999, Crole and Soley, 2011, Al-Nefeiy and Alahmary, 2015). According to our knowledge, this study recorded statistically for the first time, that the diameter of the most salivary glands in the pharynx of quail was larger than that of dove.

The present work showed that the lingual core of the root consisted of central cartilaginous plate of basihvoid bone which became ossified and surrounded by bulk of intrinsic lingual muscle. In, kestrel, owl and Eurasian hoopoe, the entoglossum extends along with tongue from tip to root and supported by skeletal muscles (Abumandour and El-Bakary, 2017b, Abumandour and Gewaily, 2019), but does not extend till apex in magellanic penguin (Spheniscus magellanicus) and kelp gull

(*Larus dominicanus*) as quoted by Samar et al., (1995). Mahmoud et al. (2019) suggested that the difference in the degree of ossification of the hyoid apparatus in birds improve the mechanical performance of the tongue.

Distribution of the caudal lingual salivary glands was varying in both dove and quail which concentrated centrally, dorsal to basihyoid of the hyoid bone in dove and dorsolaterally in quail. In this respect, the dorsal surface of the root of the tongue was characterized by a large number of the caudal lingual glands (Abou-Zaid, 2008, Farouk and Hassan, 2015, El Bakary et al., 2016, Abumandour and Gewaily, 2019). Farouk and Hassan (2015) added in dove that the most volume of root was filled glands. with lingual On contrary, Abumandour and El-Bakary (2017b) mentioned that root of the tongue in kestrel was devoid of any lingual glands and in common myna the lingual glands which located dorsolateral to the basihyoid bone are anterior one (Kadhim et al., 2013). On the other hand, these glands were absent in the lingual apex of Anas clypeata (El Bakary et al., 2016). The caudal lingual glands associated with aggregation of lymphoid tissue in dove, this result confirmed by Abumandour and El-Bakary (2019). The secretion of the lingual glands play role in rolling food on the lingual surface into esophagus as results its lubrication effect (Jackowiak and Ludwig, 2008) and protection against the pathogenic activity of microorganism (Brockhausen, 2003, Sağsöz et al., 2013).

The stratified squamous epithelium lining the laryngeal mound externally transformed into respiratory epithelium by a distance from the laryngeal inlet. Similar findings were reported in different species of birds as duck (Alsayed, 2010), goose (Mohamed et al., 2018), emu (Crole, 2011), turkey (Saleh, 2013). We added the epithelium of the median caudal part of the mound toward its larvngeal caudal commissure was keratinized in quail. micrometric analysis Moreover, our showed that the thickness of the epithelium of the laryngeal mound was thicker in quail by five times than that of dove. Concerning for the cricoarytenoid salivary glands, the current findings explained that the laryngeal mound of dove had two groups of salivary glands; rostromedial and caudomedial cricoarytenoid salivary glands. While of quail had three groups; rostromedial, rostrolateral. and caudomedial cricoarytenoid salivary glands. In duck and geese, the laryngeal mound has four groups of circoarytenoid glands (rostromedial, rostrolateral. caudomedial and caudolateral) as per the reports of Alsayed, (2010) and Mohamed et al., (2018). While in emu, the laryngeal mound has glandular and non-glandular regions, the glands located on the dorsolateral aspect of the arytenoid cartilages, and the rest of the laryngeal mound was free of glands. The laryngeal mound was supported by intrinsic and extrinsic groups of the laryngeal muscles. A similar finding was mentioned in turkey, geese (Al-Mussawy, 2012, Mohamed et al., 2018).

CONCLUSION

Collectively, the distribution of the salivary glands and aggregations of the lymphoid tissue was more in the pharyngeal cavity of quail than that of dove. Where this proves the theory that quail subjects to harmful mechanical subjects and foreign bodies, bacteria, viruses. And it may also be suggested that the amount and distribution of glandular tissue in the pharynx of quail and dove would instrument the specific diet and feeding strategy of these birds.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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