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Article

Exploring bovine fecal bacterial microbiota in the Mapimi Biosphere Reserve, Northern Mexico

Irene Pacheco-Torres [a]

Cristina García-De la Peña [b*]

César Alberto Meza-Herrera [a]

Felipe Vaca-Paniagua [c,d,e]

Clara Estela Díaz-Velásquez [c]

Claudia Fabiola Méndez-Catalá [c]

Luis Antonio Tarango-Arámbula [f]

Luis Manuel Valenzuela-Núñez [b]

Jesús Vásquez-Arroyo [g]

[a] Universidad Autónoma Chapingo. Unidad Regional Universitaria de Zonas Áridas, Bermejillo, Durango, México.

[b] Universidad Juárez del Estado de Durango. Facultad de Ciencias Biológicas, Av. Universidad s/n Fracc. Filadelfia, 35010 Gómez Palacio, Durango, México.

[c] Facultad de Estudios Superiores Iztacala. Laboratorio Nacional en Salud, Diagnóstico Molecular y Efecto Ambiental en Enfermedades Crónico-Degenerativas. Tlalnepantla, Estado de México.

[d] Instituto Nacional de Cancerología. Ciudad de México, México.

[e] Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Iztacala Unidad de Biomedicina, Tlalnepantla, Estado de México, México.

[f] Colegio de Postgraduados, Campus San Luis Potosí, Salinas de Hidalgo, San Luis Potosí, México.

[g] Universidad Juárez del Estado de Durango. Facultad de Ciencias Químicas. Gómez Palacio, Durango, México.

* Corresponding author: cristina.garcia@ujed.mx

Abstract:

In Mexico, information on the bovine fecal microbiota (*Bos taurus*) is scarce. The present study describes the diversity and abundance of bacteria in fecal samples from rangeland bovines, collected in the Mapimi Biosphere Reserve in the central part of the Chihuahuan desert. Fecal samples were analysed using high-throughput next generation massive sequencing using V3-V4 16S rRNA on Illumina Miseq. A total of 17 phyla, 24 classes, 33 orders, 50 families, 281 genera, and 297 species were identified. Firmicutes and Verrucomicrobia were the most abundant phyla. The most abundant genera were *Sporobacter,* PAC000748_g (genera into the Ruminococcaceae family) and *Eubacterium_g23*. Three genera (*Clostridium*, *Corynebacterium* and *Fusobacterium*) and one species (*Campylobacter fetus*) potentially pathogenic bovine bacteria were registered. This information represents a bacteriological baseline for monitoring the grazing bovine intestinal health status, and to trace possible interactions with the fecal microbiota of native roaming wildlife in the area.

Key words: *Bos taurus*; *Campylobacter fetus*; Bacterial diversity; 16S rRNA gene; Massive sequencing.

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Introduction

The microbial community of the gastrointestinal system of cattle remains understudied. Due to its influence on nutrient absorption, productivity, potential reservoir of human and animal pathogens, as well as overall animal health, there is a need to better understand bovine gut microbial communities(1). Recently, high-throughput sequencing using 16S rRNA amplicons has provided deeper information on the fecal bovine microbiota composition, and the results obtained to date indicate a high diversity(2).

The central part of the Chihuahuan Desert in Mexico has a high diversity of wildlife(3,4). The bovine (*Bos taurus*) has been raised as grazing livestock since its introduction at the end of the 16th century, being the most important economic activity in this area(5). However, this activity is the main reason of ecological deterioration which affects wildlife; for example, this ruminant species competes for forage resources with endemic animal species (i.e., *Gopherus flavomarginatus*, Bolson tortoise)(3). Cattle grazing also exerts strong pressure on plant populations, modifying their cover; this may increase soil erosion susceptibility in this desert(3,6).

The cattle gut microbiome has many microbial species that play an important role in health and productivity(7,8). These microbes are essential for the fermentation of consumed plant matter that is converted into energy for the host(9). However, bovines asymptomatically transport bacterial species that are potential pathogens to wildlife as *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp. and *Listeria* spp.(6,10). In recent years, the extensive use of land for agriculture has increased the densities of cattle populations creating positive correlations with pathogenic infections by fecal bacteria(11). Though, knowledge about bovine fecal bacterial diversity under grazing management systems is relatively scarce(12). This study aimed to explore for the first time the diversity and abundance of fecal bacteria from bovines under grazing-marginal conditions in the Mapimi Biosphere Reserve, center of the Chihuahuan desert, using next-generation sequencing (16S rRNA).

Material and methods

All the methods and activities of this study were in strict accordance with accepted guidelines for ethical use, care and welfare of animals in research at international(13) and national(14) levels, with institutional approval reference number UJED-FCB-2018-07.

Study area

The study was developed in the Mohovano de las Lilas locality, northeast of the Mapimi Biosphere Reserve in Mexico (26°00’ and 26°10’N, 104°10’ and 103°20’W) in the center of the Chihuahuan desert. This area has warm, very arid climate, with an average annual temperature of 25.5 ° C, and an average annual precipitation of 264 mm. The predominant vegetation is rosette and microphile scrub, as well as halophyte, and gypsophila plants(15).

Field work

In July 2018, three fresh fecal samples were collected from three healthy male bovines. From each fecal sample, 0.25 g was collected from the center of the sample and deposited it in BashingBead™ cell lysis tubes (Zymo Research Corp.) adding 750 µL of lysing/stabilizing solution. Each tube was processed in a TerraLyzer™ cellular disruptor (Zymo Research Corp.) during 20 sec according to the equipment specifications.

Laboratory work

DNA was extracted from the samples using the Xpedition™ Soil/Fecal DNA MiniPrep kit (Zymo Research Corp.) in a laminar UV flow hood in sterile conditions. The amount of DNA obtained was measured in a Qubit™ fluorometer (Invitrogen). Then, the V3-V4 region of the 16S rRNA gene was amplified using the following primers(16): S-D-Bact-0341-b-S-17, 5´-CCTACGGGNGGCWGCAG-3´ and S-D-Bact-0785-a-A-21, 5´-GACTACHVGGGTATCTAATCC-3´. The step after sequencing was realized using a Illumina protocol(17,18) and thereafter, the samples was sequenced in MiSeq of 2 × 250 paired final. The complete sequencing process is available in García-De la Peña *et al*(19).

Data availability

The files used in this study were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA614584).

Bioinformatic analysis

The DNA sequences were analyzed using Quantitative Insights into Microbial Ecology bioinformatics software (QIIME)(20). Both forward and reverse sequences were assembled using the PEAR program(21) considering Q30 the quality criterion (one false base for every 1,000 bases). Chimeric sequences were discarded with USEARCH(22). Then, the operational taxonomic units (OTUs) were selected with the UCLUST method(22) at 97 % similarity; a representative sequence for each OTU was obtained, and the taxonomy was assigned using EzBioCloud database as reference(23). A simple random rarefaction process was performed(24) in order to obtain a standardized file for all samples. The relative abundance for the phylum and family levels were represented as stacked bar plots using R, and genus level was visualized as a heatmap using Morpheus software (Morpheus, https://software.broadinstitute.org/morpheus); hierarchical clustering (average linkage method with Euclidean distance) was used to visualize samples dendrogram(25).

Results and discussion

In this study, the average number of sequences assembled was 155,915. A mean ± sd of 109,814 ± 16,686 bacterial sequences were obtained after taxonomic designation. The average number of OTUs with a 97 % of similarity was 6,661 ± 431 (Table 1).

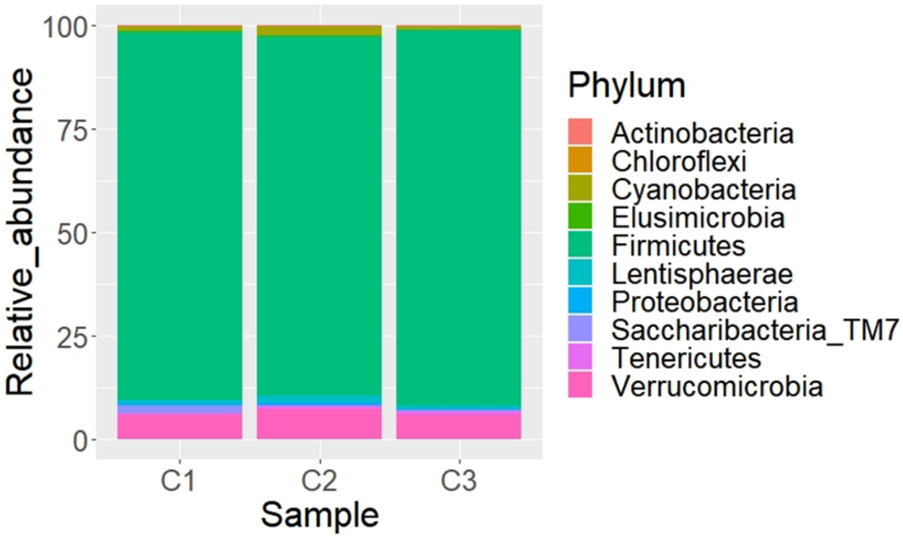
Table 1: Fecal sequences information of *Bos taurus* at the Mohovano de las Lilas locality, Mapimi Biosphere Reserve, Mexico

Sample	Total	Assembled	Discarded	BS	BSS	OTUs
1	322,428	138,862	183,566	131,275	98,084	6,293
2	223,470	145,379	78,091	136,807	102,441	6,556
3	305,380	183,506	121,874	173,177	128,916	7,135
Mean	283,759	155,915	127,843	147 086	109 814	6,661

BS= bacteria sequences after taxonomical designation, BSS= bacteria sequences after singletons removal; OTUs= operational taxonomic units.

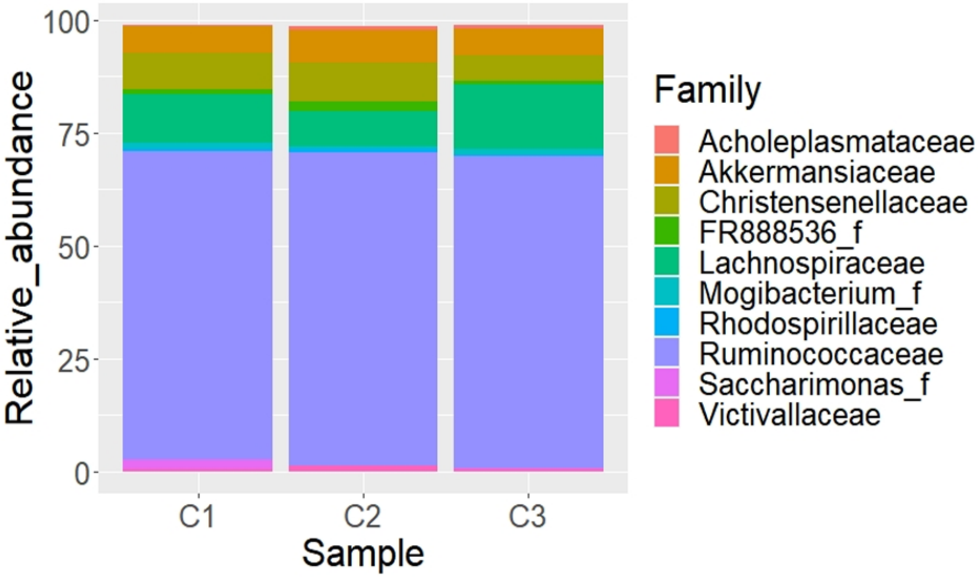
A total of 17 phyla, 24 classes, 33 orders, 50 families, 281 genera, and 297 species were determined. The most abundant phyla (Figure 1) were Firmicutes ($\bar{x} = 88.9$ %) and Verrucomicrobia ($\bar{x} = 6.4$ %). The same phyla were reported in grazing Mongolian cattle in Hulunbuir grassland and Alxa Desert in China(26). These phyla are considered normal components in the basic fecal microbiota of domestic herbivores(27,28) and other species of ruminants(29,30). Firmicutes has been reported as the most frequent phylum in fecal samples of cattle, horses(2,31,32), and red deer(33). This abundance is related to high fiber intake(34). Verrucomicrobia was the second abundant phylum in the cattle samples in this study. Aricha *et al*(26) determined that this phylum was very abundant in the intestinal tract of the grazing Mongolian cattle in the Alxa Desert, and argue that this may be related to the extremely strong disease resistance of this breed of cattle. It is important to analyze later if this phylum confers resistance to cattle diseases in the Mapimi reserve, which would represent an advantage for the bovine’s health in this area. Also, Bacteroidetes was reported in previous studies Mongolian(26), and Holstein Friesian(36) as the second most abundant phylum in other cattle species such as grazing and feedlot Angus Beef(35). However, Bacteroidetes was found in a minimum proportion (0.001%) in the cattle samples of the Mapimi reserve. This disparity can be related to the type of diet(37), geographical differences(26), and the environment in which they are distributed(38). Nevertheless, this information can only be confirmed by developing specific studies in this respect.

Figure 1: Relative abundance (%) of fecal bacteria taxa (phylum level) from three samples of *Bos taurus* at the Mohovano de las Lilas locality. Only the first 10 more abundant phyla are shown



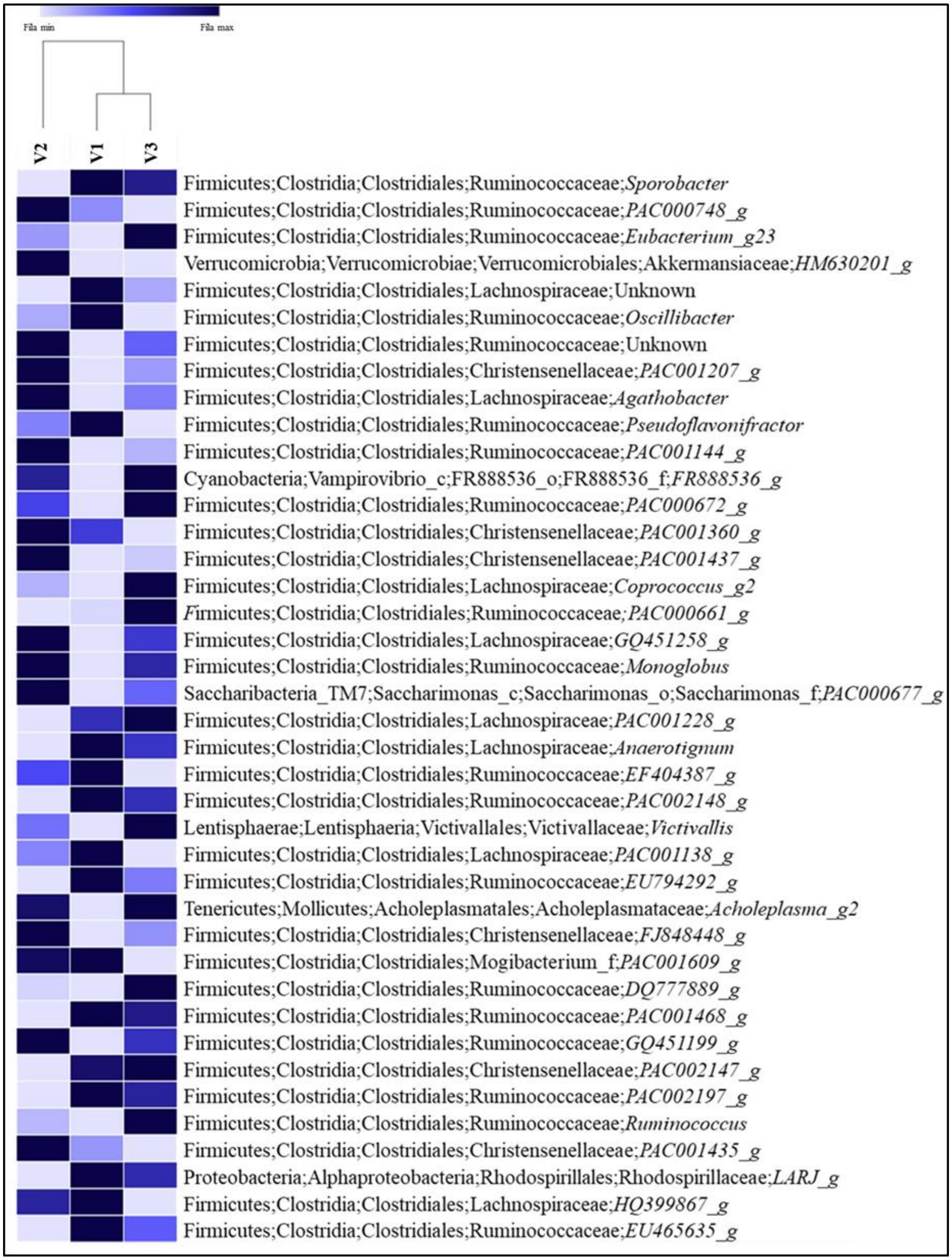
At family level, Ruminococcaceae ($\bar{x} = 68.9$ %) and Lachnospiraceae ($\bar{x} = 10.9$ %) were abundant in the fecal samples collected (Figure 2); both families are found in the mammalian gut environment and have been associated with good health(39). Some genera of the Ruminococcaceae family are part of the normal intestinal microbiota of cattle, sheep, and goats metabolizing cellulose, and colonizing the rumen(40); these bacteria taxa are important for the degradation and fermentation of polysaccharides in the diet of ruminants(41). In addition, it has been reported that members of the Lachnospiraceae family exhibit pectin hydrolysis activities in the cattle´s rumen(42) associated to the butyric acid production and providing energy for the growth of intestinal epithelial cells(43). The high abundance of Lachnospiraceae in cattle protects the intestine and acts as a barrier that favors the adaptation of the host to its environment; it also promotes a decrease in the incidence of intestinal diseases(26).

Figure 2: Relative abundance (%) of fecal bacteria taxa (family level) from three samples of *Bos taurus* at the Mohovano de las Lilas locality. Only the first 10 more abundant families are shown



From 281 classified genera found in this study, 36.6 % have a taxonomic name; this percentage is higher than the reported by Kim and Wells(44) in feces of cattle where only 110 genera were classified, and about 41 % of the total sequences couldn't be assigned to a known genus (Figure 3). Nevertheless, the results showed here increase the number of genera of the *B. taurus* fecal microbiota previously reported (12,45,46), who confirmed that the fecal bacterial microbiota is extremely diverse in cattle, and has not yet fully described. *Sporobacter* was the most abundant genus found in the fecal samples of cattle in this study. This genus was reported in alpaca(47), deer sika(28), horse(48), donkey(49), and the Bolson tortoises *Gopherus flavomarginatus*(19). This genus is related to digestion of plant ligno-cellulosic matter; however relatively little is known about the role of this bacteria in the degradation process(50). Durso *et al*(51) reported *Faecalibacterium*, *Ruminococcus*, *Roseburia*, and *Clostridium* as important components of the fecal bovine microbiota. These genera were also determined in the present study. According to some studies(52,53) these bacteria constitute 50 to 70 % of the total number of microorganisms in the digestive system of ruminants. These animals have specific gut microbial taxa as they are dependent on these bacteria to extract energy and nutrients from food(54), besides having specialized anatomical and physiological adaptations to the cellulolytic fermentation of low nutrition - high fiber vegetal material(55). The presence of other bacterial genera reported in this study could be the result of environmental and genetic factors, age, breed, diet, phylogeny, among others(56,57,58). Recently(56,59,60), was demonstrated that herbivorous animals have the most diverse microbiota since they depend on microbial metabolic pathways to maximize energy and nutrient extraction from feeding(61).

Figure 3: Heatmap of *Bos taurus* fecal bacteria sample at genus level at the Mohovano de las Lilas locality. Only the first 40 more abundant genera are shown



Although the gut microbiome usually remains stable over time assisting as a defense system against pathogens and other disease-causing agents in the host, the disturbance of this community can lead to animal disease(62,63). In the present study, the samples collected were obtained from apparently healthy bovines. However, bacteria considered of veterinary importance were found in these animals; this could be a potential health risk because they are carriers of these microorganisms. For example, *Campylobacter*, *Clostridium*, *Corynebacterium* and *Fusobacterium* were found in the fecal samples. These genera have been associated with cattle disease. *Campylobacter* has been reported as a cause of infertility and abortion in ruminants(64,65); also represents a critical threat to public health, because it can be transmitted from cattle to humans(66,67,68). *Clostridium* has been reported causing diseases and death in ruminants, especially in cattle; examples are respiratory diseases(69), botulism(70) and the blackleg(71). *Corynebacterium* has been reported in beef and dairy cattle associated with renal disease(72), mastitis(73,74), and tuberculosis(75,76,77); also, it is considered as an important emergent pathogen for humans(78). Finally, *Fusobacterium* was reported by others(79-82), causing abscesses in cattle. It is important to develop other studies that provide information on the pathogenicity and dynamics of these potential pathogens in the bovines of the Mapimi reserve.

At species level, *Pseudobacteroides cellulosolvens* and *Campylobacter fetus* were registered in the present study. *Pseudobacteroides cellulosolvens* is anaerobic bacteria that degrade plant cell wall polysaccharides and cellulosic, being capable of using cellulose or cellobiose as a sole carbon source(83). *Campylobacter fetus* is a relevant species; the main reservoirs of this bacteria are both the intestinal and the genital tracts in cattle and sheep(64,65). This species causes spontaneous abortion and infertility in cattle, while it is also an opportunistic pathogen to humans(84).

Due to the free-grazing management in the Mapimi Reserve, the bovine feces remain over the soil until natural processes degrade them. Consequently, native fauna can be in contact with these feces, increasing the probability of interspecific transmission of some bacteria(85). Although it has been previously reported that there are no evidence of cross-parasite infection between cattle and mule deer in the Mapimi Biosphere Reserve(86), it is important to clarify whether this same scenario occurs for bacteria. McAllister and Topp(87) estimate that about 77 % of the pathogens that usually infect livestock can also affect wildlife. However, also wildlife is considered an important source of microorganisms that could cause infectious diseases to domestic animals and humans(88,89). For these reasons, it is important to develop studies focused on risk management at the interface of domestic species and native fauna, considering the implications for the transmission of microorganisms with pathogenic potential(88,89). This information could lead to establish microbiological control strategies for wild fauna populations and livestock within the area.

Conclusions and implications

Information about the bovine fecal microbiota under extensive grazing conditions is scarce. From economic, ecological and health perspectives, it is crucial to determine the bacterial diversity -from phyla to species-, in the intestine of domestic ruminants. The present study is the first insight into the fecal bacterial composition of bovines in the Mapimi Biosphere Reserve in Mexico using next generation sequencing. This information significantly expands the knowledge about the composition and abundance of bacteria that are part of the microbiological community of the bovine intestine. In this case, the approach was through the analysis of feces in free grazing cattle. Although a large number of bacterial taxa were reported from the collected samples, it was not possible to determine the genus or species of some bacteria, so it is still necessary to go further into the taxonomy using specific molecular markers. However, the results obtained in the present study could be used as a bacteriological baseline for monitoring the grazing bovine intestinal health status, and to trace possible interactions with the fecal microbiota of native roaming wildlife in the area. Finally, it is important to emphasize that the next generation massive sequencing is a very effective technique that simplifies the analysis of complete bacterial communities; therefore, complementary studies on the microbiota in this and other bovine populations in Mexico are warranted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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