



Research article

Pathogen discovery in ancient cattle remains: Possible cause of taurine extinction in Thailand

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Abstract

Importance of the work: Taurine was domesticated in Thailand during the prehistoric period; however, today, zebu is the only traditional cattle species in this country. The findings of the current study suggested that the extinction of Thai taurine may have been caused by an epidemic of one or more infectious diseases.

Objectives: To identify cattle species and to reveal pathogens in ancient cattle remains in Thailand during the Dvaravati period.

Materials & Methods: Firstly, the cattle remain was identified by morphology. DNA sequencing of ancient cattle remains was performed using Illumina and then the sequences were used to identify cattle species and to classify pathogenic bacteria and viruses based on genome mapping.

Results: The cattle remain belonged to taurine or a hybrid between taurine and zebu. Moreover, several microbes causing death in cattle were detected among 26 pathogenic bacterial species and 5 pathogenic viruses that can infect cattle and other mammals, such as humans, including *Yersinia sp.* which has killed millions of humans in the past. Certain bacteria detected in this study, such as *Campylobacter sp.*, can lead to difficult pregnancies and abortion in cattle and humans.

Main finding: The cattle remain may have been from a taurine-zebu hybrid, suggesting that zebu may have been introduced into Thailand in the Dvaravati period. Several pathogens causing death in cattle, detected in the current study, have circulated in the cattle population since the Dvaravati period and some of these may have caused the extinction of taurine in Thailand.

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Introduction

Cattle domestication began approximately 10,000 years ago when humans started practicing agriculture (Svizzero and Tisdell, 2014). Currently, the two domestic cattle species are *Bos taurus* (taurine) and *Bos indicus* (zebu) (Turton, 1991). Taurine was first domesticated around 10,800 years ago in Turkey and Syria (Helmer et al., 2005). This species later spread into Europe around 8,500 years ago and into China around 5,000 years ago (Payne et al., 1997). Zebu was domesticated in the Indus Valley around 8,000 years ago and subsequently dispersed to Southeast Asia, including Myanmar, Thailand, and Vietnam, where under the influences of Buddhism and Hinduism it flourished as a part of the indigenous livestock in the region (MacHugh, 1996).

In Central Thailand, archaeological evidence, such as metal coins depicting images of humpbacked cattle believed to be zebu, has been found at various archaeological sites, including at the Khok Chang Elephant Kraal archaeological site in U Thong District, Suphan Buri province. These metal coins bear inscriptions in Sanskrit that have been dated to approximately 1,300 years ago (Boeles, 1964). Genetic evidence from cattle remains found in archaeological sites in Northeast and Central Thailand has been dated to approximately 3,500–1,700 years ago and revealed that all the cattle remains belong to taurine (Siripan et al., 2019). In contrast, the indigenous cattle breeds in Thailand are all zebu. These findings led to an investigation to determine the period when zebu cattle appeared in this region. They were possibly introduced into Thailand during the period of Indian cultural influence believed to coincide with the Dvaravati civilization.

In addition, another important issue arises regarding the extinction of taurine in Thailand. In the past, there have been events that led to the mass deaths of cattle in Europe and Africa. For example, in 1865, hundreds of millions of taurine in Europe, particularly in the United Kingdom, were killed by the morbillivirus after the importation of cattle from India (Roeder, 2011). In 1896, this virus also resulted in the deaths of over 2.5 million taurine in southern Africa, leaving only 3–7% of the original population (Kapur et al., 1994; Pearce-Duvet, 2006; Holdo et al., 2009). Only 10–30% of zebu in Africa died during the spread of the virus, indicating that zebu had higher resistance to the virus than taurine. Furthermore, domestication of taurine was substantially replaced by that of zebu after the outbreak, after which the number of zebu increased rapidly (Felius et al., 2014). It is believed that the outbreak was caused

by the introduction of cattle from India into South Africa in 1889 (Spinage, 2017).

Another epidemic disease that has contributed to the decline in cattle populations is bovine tuberculosis, caused by the bacterium *Mycobacterium tuberculosis bovis*. This disease has been present in European cattle populations for many centuries, reportedly causing emaciation and death in calves and cattle in the 1800s (Huchzermeyer et al., 1994; Morris et al., 1994; De Vos et al., 2001; Rodwell et al., 2001; Caron et al., 2003). In addition, other studies have indicated that the *M. tuberculosis* strains infecting humans evolved from *M. tuberculosis bovis* approximately 10,000 years ago, coinciding with the period when humans began domesticating cattle (Kapur et al., 1994; Baker et al., 2015), suggesting that certain infectious diseases originated in animals and then spread to humans. These pathogens cause severe health implications, with approximately 60% of all current infectious diseases in humans being of animal origin (Woolhouse and Gowtage-Sequeira, 2005). Recent study has shown that zebu has higher resistance to *M. tuberculosis bovis* than taurine (Lee et al., 2024). In addition, zebu has a higher capacity against *Brucella* species causing brucellosis disease than taurine because zebu has a higher number of monocytes and CD4⁺ T lymphocytes, which are parts of innate and adaptive immunity, respectively (Macedo et al., 2013). These findings conferred that zebu can tolerate certain pathogenic bacteria better than taurine due to highly effective immune responses.

The purpose of the current study was to identify the species of the ancient cattle remains in Central Thailand from the Dvaravati period when Indian culture was influential and cattle from India may have been introduced into Thailand during this time. Additionally, the pathogens were investigated causing diseases in cattle that could potentially lead to the extinction of taurine in Thailand. Therefore, this study should be particularly beneficial in understanding ancient animal husbandry and archaeology, shedding light on past phenomena that may have contributed to the decline of taurine populations in Thailand.

Materials and Methods

Archaeological cattle remains

Ancient cattle remains were found at the U Thong archaeological site (14°22'18.9"N 99°53'08.9"E) in U Thong District, Suphanburi Province dated approximately 1,900–1,000 years before present (YBP). At the same archaeological site,

infant skeletons were found which had died at around 36–40 weeks of gestation without clear causes of death. Infectious diseases may have been one of the factors contributing to the death (Khunsong, 2016). Many types of archaeological evidence were discovered, including inscriptions, artifacts and ecofacts from prehistoric and historic times. The evidence was mostly consistent with the Dvaravati culture, which was influenced by Indian culture (Khunsong, 2016).

One of the ecofacts found in the same layer as the infant at the U Thong site was used to identify species based on comparative morphological characteristics (Suraprasit et al., 2016; Wattanapituksakul, 2016). The bovid specimen was classified as the right mandible (377.6 g in weight and 244 mm in length). The characteristics of the teeth on the mandible include a high crown with a crescent-folded crown of molars. The specimen is considered to be *Bos sp.* which has less robust teeth than buffalo (*Bubalus sp.*). Furthermore, it is smaller and has less advanced teeth than wild cattle in Thailand (*Bos gaurus*, *B. javanicus*, *Bos sauveli*) and the size of the third molar is in the range of the recent domestic cattle.

Sample preparation and DNA extraction

The right mandible dated approximately 1,700 YBP identified as *Bos* species was cleaned with 80% ethanol and then washed with sterile water three times. Two sides of the specimens were exposed to ultraviolet light in a laminar flow for 30 min to prevent contamination from exogenous DNA. Then, the surface of the specimens was removed using a dental drill. Subsequently, the specimens were ground into a fine powder using a sterilized mortar and pestle; 0.25 g of the powder was used for DNA extraction.

DNA was extracted following the method described by Damgaard et al. (2015). Throughout DNA extraction, blank extraction was processed alongside samples to provide a negative control. The DNA concentration was measured using a Q-bit fluorometer (Invitrogen; Thermo Fisher Scientific; USA). Then, a paired-end library was prepared and sequenced using an Illumina platform (2×150 bp read length).

DNA sequence analysis

The quality of raw reads was assessed and all adapters were removed using FASTP (Chen et al., 2018). Then, the cleaned reads were mapped on two referenced genomes of *B. taurus* (NCBI: txid9913) and *B. indicus* (NCBI: txid9915) using the Bowtie2 (Langmead and Salzberg, 2012) and Samtools (Heng et al., 2009) software tools. Subsequently, to reveal the microbial diversity found in the ancient cattle remains, the sequences that did not align with the cattle genomes were taxonomically identified using the Kraken2 software (Wood et al., 2019).

Results and Discussion

Identification of *Bos* species

The right mandible (Fig. 1) from the U Thong archaeological site was identified using morphological methods. It was found that it could potentially belong to either taurine or zebu. Since conventional PCR was unsuccessful, species identification of the cattle specimen was performed using DNA obtained from Next-generation sequencing.

The number of total reads was 65,295,458 (x2) and these sequences were aligned with the genomes of two cattle species (taurine and zebu) to identify the species of the ancient cattle remains. Based on the results, 460 reads were aligned one time to taurine or zebu (Table 1), with 300 reads (65.22%) mapped to taurine and 160 reads (34.78%) mapped to zebu.



Fig. 1 Cattle mandible aged approximately 1,700 years ago from U Thong Archaeological Site and used in current study, where black and white gradations on scale bar indicate 1 cm

Table 1 Number of DNA sequences obtained from ancient cattle aligned with two cattle genomes (*Bos taurus* and *Bos indicus*)

Referenced genome	Strand	Total read	Aligned 0 times	Aligned 1 time	Aligned >1 times	Overall alignment rate
<i>Bos taurus</i>	R1	65295458	65294632	198	628	0.00%
<i>Bos taurus</i>	R2	65295458	65292040	102	3316	0.01%
<i>Bos indicus</i>	R1	65295458	65294876	64	518	0.00%
<i>Bos indicus</i>	R2	65295458	65294134	96	1,228	0.00%

In total, 5,690 reads were aligned more than one time to cattle sequences, with 3,944 reads (69.31%) mapped to taurine and 1,746 reads (30.69%) mapped to zebu, indicating that the cattle specimen in the current study was more closely related to taurine than zebu. The findings suggested that the ancient cattle may have belonged to taurine or a hybrid between taurine and zebu. Even though all of the ancient Thai cattle in another study dated approximately 3,500–1,700 YBP belonged to taurine, only a partial D-loop in mitochondrial DNA was analyzed which cannot detect any admixture between two cattle species (Siripan et al., 2019). In addition, hybrids between these two cattle species have been found in many regions of the world (MacHugh 1996; Felius et al., 2014); thus, it was possible that hybridization may have also occurred in Thailand. Additionally, the age of the cattle remains from the U Thong archaeological site was approximately 1,700 YBP, suggesting that taurine and zebu may have been domesticated together in Central Thailand during the

Dvaravati period and that zebu may have been introduced into Thailand at least since 1,700 YBP.

Classification of pathogens causing diseases in cattle

Approximately 130 Mb sequences that did not align with the cattle genomes were examined and classified. This dataset included 7,704,369 sequences that were identified as bacteria. Of these, 7,340 sequences were identified belonging to 26 bacterial species in 19 genera that can cause diseases in cattle and other mammals, including humans (Table 2). Among them, six species can specifically infect and cause diseases only in cattle. Additionally, one bacterial species, *Paenibacillus bovis* can infect cattle but does not cause any disease (Xu et al., 2016). Furthermore, 718 sequences of viruses were identified, of which five can cause diseases in cattle and other mammals. Among them, two species can specifically infect and cause diseases only in cattle (Table 2).

Table 2 Pathogenic bacteria and viruses detected in ancient cattle remains

Pathogen	No. read	Diseases	Reference
Bacteria			
<i>Yersinia</i> sp.	372		
<i>Yersinia pseudotuberculosis complex</i>	171	Yersiniosis	Savin et al. (2014)
<i>Yersinia pseudotuberculosis</i>	100	Yersiniosis	Slee et al. (1988)
<i>Yersinia enterocolitica</i>	101	Yersiniosis	Schaake et al. (2013)
<i>Mycobacterium</i> sp.	398		
<i>Mycobacterium tuberculosis</i>	243	Tuberculosis	Buendía et al. (2013)
<i>Mycobacterium tuberculosis bovis</i> *	155	Tuberculosis	Kapur et al. (1994)
<i>Bacillus anthracis</i>	3	Anthrax	Fasanella et al. (2010)
<i>Staphylococcus aureus</i>	165	Mastitis	Gomes and Henriques (2016)
<i>Listeria monocytogenes</i>	6	Abortion, Listeriosis	Woo-Sam (1999)
<i>Clostridium</i> sp.	685		
<i>Clostridium septicum</i>	646	Malignant edema	Wright (2016)
<i>Clostridium botulinum</i>	30	Botulism	Animal Biosecurity and Welfare (2018)
<i>Clostridium perfringens</i>	8	Enterotoxemia	Wright (2016)
<i>Clostridium chauvoei</i>	1	Black leg	Wright (2016)
<i>Brucella</i> sp.	868	Brucellosis	Tulu (2022)
<i>Pseudomonas aeruginosa</i>	3439	Mastitis	Gomes and Henriques (2016)
<i>Klebsiella pneumoniae</i>	463	Mastitis	Gomes and Henriques (2016)
<i>Escherichia coli</i>	179	Mastitis, Gastrointestinal diseases	Gomes and Henriques (2016) Peek et al. (2018)
<i>Proteus mirabilis</i>	1	Mastitis	Gomes and Henriques (2016)
<i>Pasteurella multocida</i>	37	Neonatal mortality	Doyle-Baker et al. (2020)
<i>Helicobacter pylori</i>	12	Gastrointestinal diseases	Kargar et al. (2012)
<i>Campylobacter</i> sp.	10		
<i>Campylobacter fetus</i>	2	Campylobacteriosis	Hoffer (1981)
<i>Campylobacter jejuni</i>	8	Abortion	Uzal (2022)

Table 2 Continued

Pathogen	No. read	Diseases	Reference
<i>Salmonella</i> sp.	579	Gastrointestinal diseases	Upatum et al. (1983)
<i>Mycoplasma</i> sp.	15		
<i>Mycoplasma bovis</i> genitium*	14	Subfertile	Parkinson (2019)
<i>Mycoplasma bovis</i> *	1	Mastitis, Respiratory disease	Dudek et al. (2020)
<i>Bartonella bovis</i> *	1	Angiomatosis, Peliosis hepatis	Dahmani et al. (2017)
<i>Moraxella bovoculi</i> *	8	Pinkeye	McMullen et al. (2017)
<i>Pajaroellobacter abortibovis</i> *	270	Epizootic bovine Abortion	Brooks et al. (2016)
Virus			
Suid alphaherpesvirus 1	1	Aujeszky's disease	Passler et al. (2012)
<i>Orthobunyavirus</i> sp.	1	Central nervous system disease	Dutuze et al. (2022)
<i>Circovirus</i> sp.	1	Respiratory disease, Abortion	Nayar et al. (1999)
Bovine alphaherpesvirus 1*	4	Reproductive disease, Encephalitis	Graham (2013)
Bovine alphaherpesvirus 5*	1	Encephalitis	Fenner (2017)

* = pathogen can specifically infect cattle.

Pathogenic bacteria

From the taxonomic classification, there were 26 bacterial species causing diseases in cattle that can be divided into four groups based on the source of the diseases that they cause: gastrointestinal tract, respiratory tract, reproductive system, and others.

Diseases affecting the gastrointestinal tract can have detrimental effects on growth and reproductive fitness (Peek et al., 2018). In this study, five bacterial species—*Helicobacter* sp., *Salmonella* sp., *Yersinia* sp., *Clostridium perfringens* and *Escherichia coli*—were detected in the cattle remains and they can cause gastrointestinal diseases in cattle and humans (Peek et al., 2018). Of these five, *H. pylori* is a bacterium that can adapt to several host organisms and lead to gastric cancer (Atherton and Blaser, 2009). *H. pylori* in cattle and sheep have shown genetic similarities with *H. pylori* found in humans, suggesting that this bacterium can be transmitted from animals to humans (Franceschi et al., 2014). Another bacterium, *Salmonella* sp. often causes severe infections in cattle aged less than 12 wk, with pregnant cows being at higher risk of infection (Gibson, 1965; Smith et al., 1979). Cattle infected with *Salmonella* sp. can suffer from hemorrhagic gastroenteritis and bleeding in various organs, such as the kidneys and intestines, which can lead to death (Upatum et al., 1983). Recently, the results were reported of an investigation into the sudden deaths of humans dated to approximately 3,000 YBP at the Xinjiang archaeological site in northern China located in the Proto-Silk Road between East Asia and Europe (Wu et al., 2021). The genetic analysis of the human remains revealed the presence of *Salmonella*

enterica in 6 out of 14 skeletons. No clear physical signs of the cause of death were evident, suggesting the possibility of infectious diseases. In addition, Yersiniosis is another important disease affecting the gastrointestinal system, with *Yersinia enterocolitica* causing inflammation of the intestines, acute enteritis and diarrhea (Bottone, 1997). Neumann et al. (2022) reported that human skeletal remains on the Island of Crete in Greece during the Late Neolithic to the Bronze Ages exhibited infections of *S. enterica* and *Yersinia* sp. These findings supported that these bacteria affecting the gastrointestinal tract had caused deaths in humans since prehistoric periods.

Diseases affecting the respiratory tract can spread rapidly, making respiratory infections a major concern (Chompuchan et al., 1996). In the current study, three bacterial species causing respiratory diseases were detected: *Y. pseudotuberculosis*, *Klebsiella pneumonia* and *Mycobacterium tuberculosis* and *Mycobacterium tuberculosis bovis*, of which the latter can specifically infect cattle. Presently, there are nine *Yersinia* sp., with three of them (*Y. pseudotuberculosis*, *Y. enterocolitica* and *Y. pestis*) known to infect both animals and humans (Schaaake et al., 2013). *Y. pestis* evolved from *Y. pseudotuberculosis* around 7,000 years ago and it was responsible for the Black Death that killed more than 50% of the European population during the 14th century (Benedictow, 2021; Susat et al., 2021). Another disease, tuberculosis, is caused by *M. tuberculosis* which can affect bones, the central nervous system and other organs in humans. Kapur et al. (1994) suggested that *M. tuberculosis* evolved from *M. tuberculosis bovis*, which circulated in cattle approximately 10,000 years ago. This timeframe coincides with the period when humans began domesticating cattle (Baker et al., 2015).

Lastly, *K. pneumoniae* can cause pneumonia and mastitis in cattle, with mastitis capable of leading to the spread of the infection to calves via the infected milk, resulting in death before weaning (Munoz et al., 2006). These findings support the claim that certain pathogenic bacteria in cattle have evolved and spread to humans. Furthermore, some bacteria affecting the respiratory system, especially *Yersinia sp.*, can cause a large culling of human populations (such as the Black Death plague noted above), so it is likely that it may also decrease cattle populations and possibly cause the extinction of susceptible cattle species.

In total, 11 bacterial species were identified in the current study that cause diseases in the reproductive system and have major impacts on the fertility of mammals: *Brucella sp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Campylobacter fetus*, *Campylobacter jejuni*, *Mycoplasma bovis*, *Mycoplasma bovis* and *Pajaroellobacter abortibovis*. Among these, *M. bovis*, *M. bovis* and *P. abortibovis* can specifically infect cattle. Out of these 11 species, 6 (*S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *M. bovis* and *P. mirabilis*) can cause mastitis which severely affects cattle herds, especially newborn calves leading to a decrease in survival rates (Gomes and Henriques, 2016). *Brucella sp.* causes Brucellosis or contagious abortion, which can be transmitted to other milk-producing animals, including humans (Department of Livestock Development, 2023). Three *Brucella sp.* (*B. melitensis* from goats and sheep, *B. suis* from pigs, and *B. abortus* from cattle) can cause diseases in humans and in fact affect an estimated 500,000 people annually (Moreno, 2014; Hull and Schumaker, 2018). *Brucella sp.* has been historically prevalent in regions around the Mediterranean Sea, particularly during the Roman and Medieval periods (D’Anastasio et al., 2011). In addition, campylobacteriosis or vibriosis is a disease caused by the bacterium *Campylobacter sp.* that can lead to difficult pregnancies, calf mortality and abortion (Garcia et al., 1983). *C. fetus* can also infect humans, primarily pregnant women (Wagenaar et al., 2014). These findings support that bacterial-caused diseases in the reproductive system can affect cattle and human populations by decreasing the survival rates of newborns. Several infectious agents causing abortion have been discovered in ancient cattle remain alongside an infant skeleton at the same burial site (Khunsong, 2016). Hence, pathogens causing diseases in a reproductive system may be a factor contributing to the death of the infant.

Besides the three groups of bacteria identified in the current study, there are other bacteria causing diseases in other systems. One such bacterium is *Bacillus anthracis*, which causes anthrax (World Health Organization and International Office of Epizootics, 2008). This disease can be transmitted to humans, leading to global outbreaks (Fasanella et al., 2010). Acute anthrax in cattle is characterized by shaking and difficulty in breathing with convulsions, with fatal consequences being common (Redmond et al., 1997; O’Rourke, 2001). Another severe disease is blackleg, caused by *Clostridium chauvoei*, which is commonly found in the intestines of cattle, from where it enters the bloodstream and spreads throughout the body, with infected animals dying within 12–48 hr (Wright, 2016). These findings indicate that these two bacterial species can kill cattle in a short period of time.

Pathogenic viruses

Based on the results from the current study, five viruses were identified causing diseases in cattle and other mammals, with two of them—bovine alphaherpesvirus 1 (BoHV-1) and bovine alphaherpesvirus 5 (BoHV-5) being specifically associated with cattle as hosts (Table 2). BoHV-1 causes infectious bovine rhinotracheitis that is transmitted through the air and infects the respiratory mucosa and tonsils (Aschner and Herold, 2021). Most animals show mild or no symptoms and can recover within 4–5 d unless there are complications caused by bacterial infections in the lungs that can lead to severe symptoms and death (Chompuchan, 1996). If the infection spreads to the reproductive system in pregnant cattle, the infection can lead to abortion, while if young calves become infected, severe symptoms such as fever, diarrhea, staggering, seizures, and death, may occur within a short period (Aschner and Herold, 2021). BoHV-5 is associated with bovine encephalitis fever, which is recognized as a severe form of viral encephalitis in calves (Carrillo et al., 1983; Perez et al., 2002; Vogel et al., 2003). These findings support that these viruses can cause a reduction in cattle population by killing calves.

Other genetic studies have indicated that only taurine had been domesticated in Thailand during the prehistoric period (3,500–1,700 YBP); however, today the only traditional cattle species in the country is zebu (Wangkumhang et al., 2015; Siripan et al., 2019). Thus, zebu was introduced into Thailand later than taurine, with the latter now extinct in Thailand. These facts raise two important issues—the extinction of taurine in Thailand and the timing of zebu introduced into the country. The current study focused on the first issue.

Several pathogens detected in the current study have caused mass deaths and some have circulated in cattle since prehistoric times. Certain bacteria can infect both cattle and humans, such as *C. fetus*, *H. pylori* and *Y. pseudotuberculosis*, with the latter having caused mass human deaths in Europe during the 14th century (Susat et al., 2021). There is evidence that taurine had been substantially replaced by zebu after an endemic of an infectious disease during which more than 90% of taurine had died, while more than 70% of zebu survived (Feliuss et al., 2014). Furthermore, the event occurred after the importation of zebu. In addition, other studies have demonstrated that zebu has higher resistance to several pathogens, such as *Brucella sp.* and *M. tuberculosis bovis*, than taurine due a more effective immune response (Macedo et al., 2013; Lee et al., 2024). It is possible that after zebu-carried pathogens had been introduced, the infectious diseases spread and caused mass deaths of taurine populations. These findings would support that infectious agents may be one of the important factors that caused the extinction of taurine in Thailand after zebu had been introduced.

In summary, today, zebu is only the indigenous cattle species in Thailand. However, based on the results of the current study, the ancient cattle remain may belong to taurine or possibly a hybrid between taurine and zebu, suggesting that taurine and zebu may have been domesticated together in Central Thailand during the Dvaravati period, with zebu from India perhaps being introduced into Thailand at least 1,700 YBP. Zebu has higher resistance to several pathogenic bacteria and viruses than taurine. In the past, the spread of certain pathogens could have killed millions of taurine in Africa and Europe after the importation of zebu. The current study investigated cattle remains from an archaeological site in Thailand and detected the DNA of zebu and several pathogens that can cause death in cattle. Some of these pathogens, such as *Yersinia sp.*, have been responsible for historical widespread mortality in humans. Hence, it is possible that a similar phenomenon may have occurred in other regions, including Thailand, where specifically, infectious agents may have caused the extinction of taurine in the country after zebu from India had been imported.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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