



**HAL**  
open science

## Dairy pastoralism sustained Eastern Eurasian steppe populations for 5000 years

Shevan Wilkin, Alicia Ventresca Miller, William T T Taylor, Bryan K Miller, Richard W Hagan, Madeleine Bleasdale, Ashley Scott, Sumiya Gankhuug, Abigail Ramsøe, Christian Trachsel, et al.

► **To cite this version:**

Shevan Wilkin, Alicia Ventresca Miller, William T T Taylor, Bryan K Miller, Richard W Hagan, et al.. Dairy pastoralism sustained Eastern Eurasian steppe populations for 5000 years. *Nature Ecology & Evolution*, 2020, 4 (3), pp.346-355. 10.1038/s41559-020-1120-y . hal-03029810

**HAL Id: hal-03029810**

**<https://hal.science/hal-03029810v1>**

Submitted on 29 Nov 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Dairy pastoralism sustained Eastern Eurasian steppe populations for 5000 years

Shevan Wilkin<sup>1</sup>, Alicia Ventresca Miller<sup>1,2</sup>, William T.T. Taylor<sup>1,3</sup>, Bryan K. Miller<sup>1,4</sup>, Richard W. Hagan<sup>5</sup>, Madeleine Bleasdale<sup>1</sup>, Ashley Scott<sup>5</sup>, Sumiya Gankhuyg<sup>6</sup>, Abigail Ramsøe<sup>7,8</sup>, Christian Trachsel<sup>9</sup>, Paolo Nanni<sup>9</sup>, Jonas Grossmann<sup>9</sup>, Ludovic Orlando<sup>10,11</sup>, Mark Horton<sup>12</sup>, Philipp Stockhammer<sup>5,13</sup>, Erdene Myagmar<sup>6</sup>, Nicole Boivin<sup>1,14,15,16</sup>, Christina Warinner<sup>5,17,18</sup>, Jessica Hendy<sup>1,5,7</sup>

1. Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, 07745, Germany
2. University of Michigan, Department of Anthropology, Ann Arbor, Michigan, 48109, USA
3. University of Colorado, Department of Anthropology, Museum of Natural History, Boulder, CO, 80309-0233, USA
4. University of Oxford, Faculty of History, Oxford, OX1 2BE, UK
5. Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena, 07745, Germany
6. Anthropology and Archaeology Department, National University of Mongolia, Ulaanbaatar, 14201, Mongolia
7. BioArCh, Department of Archaeology, University of York, York, YO10 5NG, UK.
8. Department of Earth Sciences, Natural History Museum, London, SW7 5BD, UK
9. Functional Genomics Centre, University of Zürich/ETH, Zürich, 8057, Switzerland
10. Laboratoire d'Anthropobiologie Moléculaire et d'Imagerie de Synthèse, CNRS UMR 5288, Université de Toulouse, Université Paul Sabatier, 31000 Toulouse, France
11. Globe Institute, Faculty of Health and Medical Sciences, University of Copenhagen, 1350K Copenhagen, Denmark
12. School of Arts, University of Bristol, Bristol, BS8 1UU, UK
13. Institut für Vor- und Frühgeschichtliche Archäologie und Provinzialrömische Archäologie, Ludwig-Maximilians University, Munich, 80799, Germany
14. School of Social Science, The University of Queensland, Brisbane, QLD 4072, Australia
15. Department of Anthropology and Archaeology, University of Calgary, 2500 University Dr. N.W., Calgary, AB, T2N 1N4, Canada
16. Department of Anthropology, National Museum of Natural History, Smithsonian Institution, 10th St. & Constitution Ave. NW Washington, D.C. 20560, USA
17. Institute for Evolutionary Medicine, University of Zürich, Zürich, CH-8057, Switzerland
18. Department of Anthropology, Harvard University, Cambridge, MA 02138, USA

## Abstract

Dairy pastoralism is integral to contemporary and past lifeways on the eastern Eurasian steppe, facilitating survival in agriculturally challenging environments. While previous research has indicated that ruminant dairy pastoralism was practiced in the region by *c.* 1300 BCE, the origin, extent and diversity of this custom remains poorly understood. Here we analyze ancient proteins from human dental calculus recovered from geographically diverse locations across Mongolia and spanning 5,000 years in time. We present the earliest evidence for dairy consumption on the eastern Eurasian steppe by *c.* 3000 BCE, and the later emergence of horse milking at *c.* 1200 BCE, concurrent with the first evidence for horse riding. We argue that ruminant dairying contributed to the demographic success of Bronze Age Mongolian populations, and that the

46 origins of traditional horse dairy products in eastern Eurasia are closely tied to the regional  
47 emergence of mounted herding societies during the late second millennium BCE.

48

## 49 **Main**

50

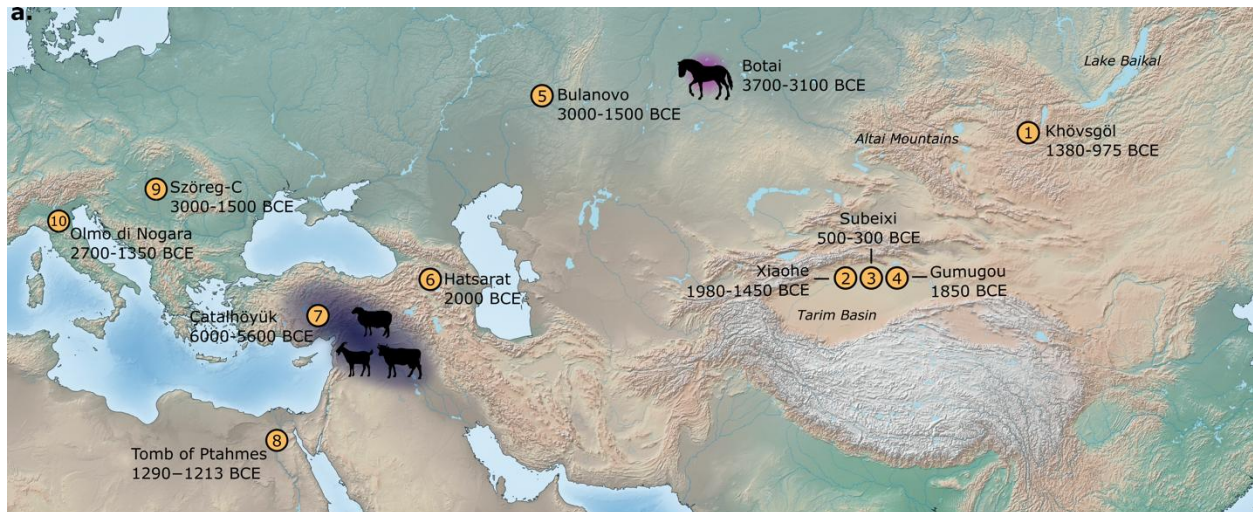
51 In contemporary central and eastern Eurasia, mobile dairy-based pastoralism is a key subsistence  
52 practice for many people<sup>1</sup>. Much of the eastern Eurasian Steppe is covered by dryland grasses  
53 which, while challenging for grain agriculture, can sustain large meat and dairy-producing  
54 herds<sup>2,3</sup>. Across the steppe, dairy is a staple food and the product of a rich culinary tradition. In  
55 the Mongolian countryside, fresh, fermented, processed and distilled dairy products provide a  
56 major source of hydration and up to 50% of summer caloric intake<sup>1,4</sup>. Moreover, milk provides a  
57 protein- and fat-rich dietary component, while the processing of milk into dairy products enables  
58 the creation of a storable and transportable food source. In Mongolia today, dairy livestock,  
59 including sheep, goat, horse, cow, yak, reindeer, and camel, are exploited for milk, meat, traction  
60 and transport across diverse environmental niches.

61

62 The adoption of dairy foods into adult human diets was a major transition in prehistoric  
63 subsistence<sup>2</sup>, and in Western Eurasian contexts, biomolecular approaches have been extensively  
64 applied to investigate its archaeological antiquity. Following their initial domestication in  
65 Southwestern Asia, cattle (*Bos taurus*), sheep (*Ovis aries*) and goats (*Capra hircus*) spread  
66 eastward across the Eurasian steppe into Central Asia<sup>5</sup>. Biomolecular evidence for dairy lipids  
67 has been identified in ceramics from Neolithic Anatolia<sup>6</sup> and eastern Europe<sup>7</sup>, as well as Copper  
68 and Bronze Age Kazakhstan<sup>8,9</sup>, indicating the potential spread of dairying out of southwestern  
69 Asia. As far east as the Tarim Basin of the Xinjiang region in northwest China in the middle  
70 Bronze Age, milk proteins have been identified in a woven basket<sup>10</sup> and pieces of well-preserved  
71 kefir cheese<sup>11</sup> (Figure 1). In Bronze Age Mongolia, a recent study of human dental calculus from  
72 individuals across multiple sites in the northern Khövsgöl aimag identified milk proteins from  
73 sheep, goat, and cattle<sup>1</sup>. Ancient DNA analysis of the same population found that almost all  
74 individuals were of predominantly local ancestry, and only a single individual had over 10% of  
75 Western steppe herder (WSH) ancestry. This suggests that by the late second millennium BCE,  
76 dairy pastoralism had been fully adopted by, or originated with, local northern populations,  
77 leaving an open question of when and how dairy subsistence arrived in this region.

78

79



80  
 81 **Figure 1. Ruminant and equine dairying in prehistoric Eurasia and contemporary**  
 82 **Mongolia.** (a) Map of Eurasia showing major geographical features referred to in the text and  
 83 sites where evidence of dairying has been previously found using proteomic approaches: (1)  
 84 Khövsgöl<sup>1</sup>, (2) Xiaohe<sup>11</sup>, (3) Gumugou<sup>10</sup>, (4) Subeixi<sup>12</sup>, (5) Bulanovo<sup>13</sup>, (6) Hatsarat<sup>13</sup>, (7)  
 85 Çatalhöyük West<sup>14</sup> (8), Tomb of Ptahmes<sup>15</sup>, (9) Szöreg-C (Sziv Utca)<sup>13</sup>, (10) Olmo di Nogara<sup>13</sup>.  
 86 Locations for the earliest evidence of ruminant dairying based on the presence of milk fats in  
 87 ceramics is shown in blue<sup>6</sup> and the earliest evidence of horse dairying<sup>8</sup> shown in pink. (b-f)  
 88 Mongolian dairy products from Khövsgöl aimag and (g) dairying rituals from Dundgobi aimag,  
 89 Mongolia. (b) Yogurt starter culture, Khöröngö (Хөрөнгө); (c) curd from reindeer milk, ‘kurd’;  
 90 (d) dried curd from mixed yak and cow milk, aaruul (ааруул); (e) clotted cream from mixed yak  
 91 and cow milk, öröm (өрөм); (f) fermented horse milk, airag (айраг); and (g) blessing ritual for  
 92 the first horse airag production of the season.

93  
 94 Direct evidence into the timing and nature of pastoral economies in Mongolia from other datasets  
 95 is exceedingly rare. On the Eastern Steppe, the ephemeral nature of pastoral campsites and  
 96 severe wind deflation in most contexts makes detecting occupational sites with direct  
 97 information on subsistence economies challenging<sup>28</sup>. As a result, archaeologists have often been  
 98 forced to form conclusions about local subsistence from materials found in ritual human burials  
 99 under stone monuments that dominate the Bronze Age archaeological landscape, and which  
 100 occasionally include satellite animal burials. Specific features of burial mounds (stone type,  
 101 shape, ringed fences) can be used to identify interred individuals into different culture groups as  
 102 mound construction styles changed alongside evolving cultural traditions in Bronze Age  
 103 Mongolia<sup>2</sup>. Prior to the Bronze Age, before the presence of constructed stone burial mounds,  
 104 there are very few uncovered occupation or ritual sites, and pre-Bronze Age subsistence

105 strategies are not well understood. However, it is assumed that Neolithic subsistence strategies  
106 included hunting, gathering, and fishing, although the possibility of pastoralism should not be  
107 completely discounted<sup>8</sup>. Human burials associated with Afanasievo and Chemurchek culture  
108 groups (c. 3000-2500 BCE) contain faunal remains of ovicaprid, bovine, equid, and dog  
109 remains<sup>9-12</sup>, yet it is unclear whether the remains of each were of a domesticated variety or their  
110 wild relatives, such as *Ovis ammon* and *Capra ibex* in the case of ovicaprids<sup>13</sup>. Later Bronze Age  
111 (1500-800 BCE) campsites containing ruminant and equine remains suggest the dietary  
112 consumption of horses and cattle, as well as sheep and/or goats as part of a fully pastoral  
113 economy<sup>14</sup>. Satellite burials containing multiple animals surrounding ritual human internments  
114 attest to pastoral culling and herd management patterns of sheep, goat, cattle, and horses during  
115 this period<sup>15,16</sup>. By 1200 BCE, remains of domesticated horses became almost ubiquitous at ritual  
116 burials sites in Northern Mongolia, with some of the crania displaying evidence for equine  
117 dentistry and horse bridling and riding<sup>17,18</sup>.

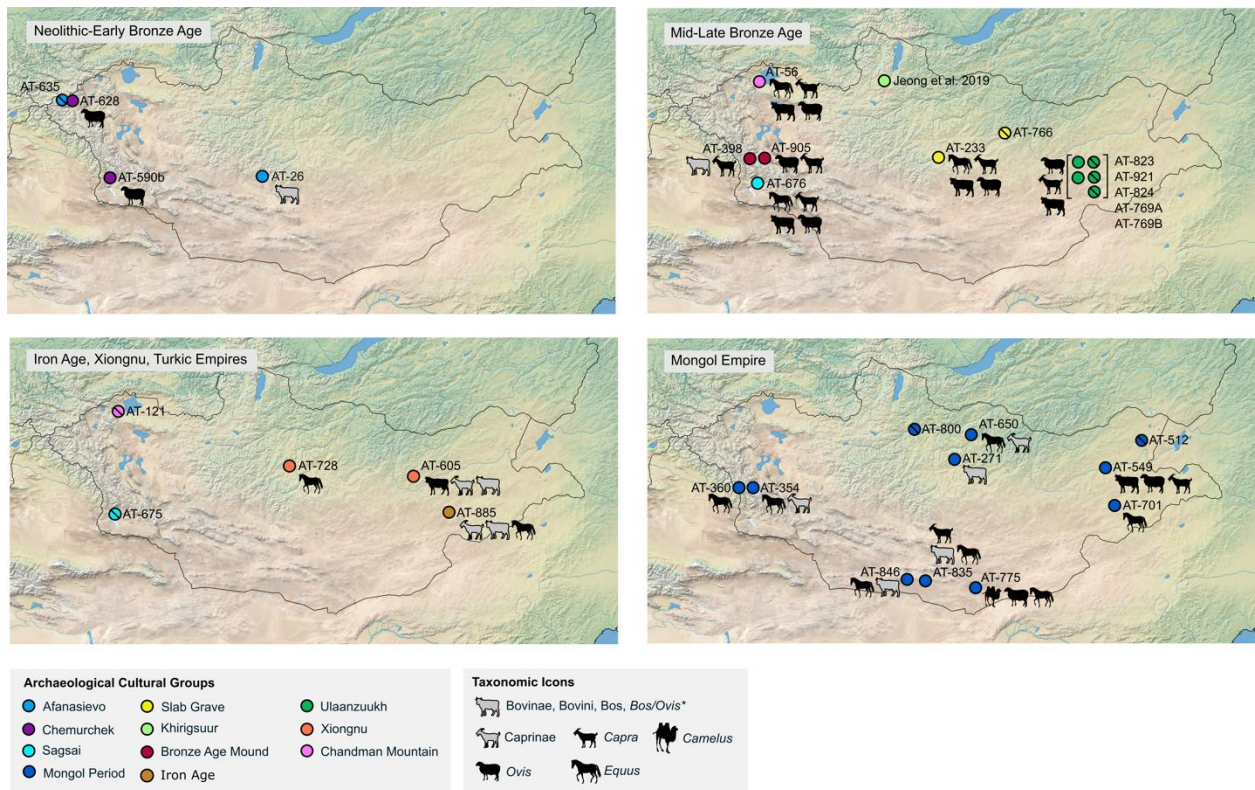
118  
119 In later time periods, written records from neighboring regions, such as China and the Middle  
120 East, as well as within Mongolia, document the importance of ruminant and equine dairy in day-  
121 to-day subsistence, particularly the consumption of fermented horse milk as early as the Xiongnu  
122 Empire (c. 200 BCE - 100 CE)<sup>19</sup> along with camel milk by the Mongol period (c. 1206-1398  
123 CE)<sup>20,21</sup>. Even though many archaeologists and historians assume milk had been included in  
124 ancient steppe diets, little direct evidence has been available about where and when specific  
125 animal species were first exploited for dairy on the steppe east of the Altai Mountains.

126  
127 The analysis of ancient proteins extracted from ancient human dental calculus (calcified dental  
128 plaque or tooth tartar) has been established as an approach for detecting milk consumption in  
129 past individuals<sup>22-24</sup>. Differences in amino acid sequences between taxa enable the detection of the  
130 livestock species, enabling ‘zooarchaeology by proxy’ - a method by which to detect past animal  
131 use by the analysis of human remains alone. Here we apply the proteomic analysis of ancient  
132 dental calculus to 32 individuals spanning the late Neolithic through the Middle Ages to  
133 characterize the antiquity and species diversity of ruminant and equine dairying in Mongolia. We  
134 report the earliest direct evidence for dairy consumption in East Asia (east of the Altai  
135 Mountains), finding that ruminant dairy consumption was a feature of ancient diets in Mongolia  
136 from its initial pastoral occupation, c. 3000 BCE and occurred in association with archaeological  
137 sites linked to western steppe cultures. We show that ruminant dairying became widespread by  
138 the Middle Bronze Age (1800-1200 BCE), and then from 1200 BCE we observe the onset of  
139 horse milk consumption, perhaps the progenitor of the alcoholic drink airag, in tandem with the  
140 first evidence for mounted horseback riding and highly mobile economies – a tradition that  
141 remained significant through the great nomadic empires and into the modern era.

## 142 143 **Results**

144 Milk proteins were identified in 72% of individuals analyzed (23 of 32 individuals, Table 1),  
145 indicating the widespread consumption of dairy foods across multiple time periods in prehistoric  
146 and historic Mongolia. Specifically, we detected evidence of the milk proteins beta-lactoglobulin  
147 (I and II), alpha-S1-casein, kappa-casein, alpha-lactalbumin and beta-casein, lysozyme C, and  
148 peptidoglycan recognition protein 1. Beta-lactoglobulin (BLG) was the most frequently detected  
149 milk protein, a pattern consistent with previous observations from ancient dental calculus<sup>22,25</sup>. We  
150 observe evidence of milk consumption from diverse taxa across multiple environmental zones

151 within Mongolia (Figure 2). Of the seven dairy livestock species utilized in contemporary  
 152 Mongolia, we identified milk peptides from goat, sheep, cattle, horse and camel, but did not  
 153 detect peptides that could be assigned specifically to reindeer or yak milk.  
 154



155 **Figure 2. Mongolian dairy consumption by period: (a)** Neolithic to Early Bronze Age; **(b)**  
 156 **Middle-Late Bronze Age; (c)** Iron Age and Early Medieval; **(d)** Late Medieval. Archaeological  
 157 site cultural affiliation is indicated by color. Solid filled circles indicate individuals with positive  
 158 evidence of milk proteins, individuals of the same site are contained within brackets; no milk  
 159 proteins were detected at sites bisected by a diagonal line. Individual AT-923, associated with  
 160 Ulaanzuukh, is not directly radiocarbon dated and is not included in this figure. Taxonomic icons  
 161 only indicate the most specific taxa identified in a phylogenetic branch. The full list of dairy  
 162 species identified for each individual is listed in Table 1.  
 163  
 164

165 **Table 1. Presence of dairy proteins by individual and site.** Radiocarbon dates are calibrated to  
 166 a 2-sigma probability (Supplementary Table 6). Bovinae/Ovis\* identifications refer to the  
 167 detection of a polymorphic amino acid position where in the identified peptide  
 168 TPEVD(D/N/K)EALEK, D is specific to Bovinae, N is specific to *Ovis* and K is specific to  
 169 *Capra*. Because asparagine (N) deamidates to aspartic acid (D), the presence of a D at this  
 170 position cannot be unambiguously assigned to Bovinae or *Ovis*. References to archaeological  
 171 sites are; 1 Novgorodova, E.A. 1989 Drevniaia Mongoliia. Nekotorye porblrmy khronologii i  
 172 etnokul'turnoi istorii. Moscow; 2. Excavated by Kovalev and Erdenebaatar, International Central  
 173 Asian Archaeological Expedition<sup>2</sup>; 3 Excavated by Tumen, Khatanbaatar, and Erdene<sup>26</sup>; 4  
 174 Excavated by D. Erdenebaatar, 2005, Mongolian Archaeological Expedition of Central Asian  
 175 Mongolia. National University of Mongolia samples records; 5. Excavated by Munkhbayer in  
 176 2012, north of Berkh Mountain. National University of Mongolia sample records; 6. Excavated

177 by Tumen, Nomin, and Volkov in 1979; 7. Excavated by D. vanaan, and Lhagvasuren in 1987.  
 178 Khovd Mankhan. National University of Mongolia sample records; 8. Excavated by Eregzen,  
 179 Mongolian Academy of Sciences<sup>2</sup>; 9. Excavated by M. Erdene, and D. Navaan in 2008, as part  
 180 of the AA-I expedition in Ulaanbaatar, Khan-Uul District, XIII Songino, Khaikhan District; 10.  
 181 Khatanbaatar, D. (2007). Tamiryn Ulaan Khoshuun dakh' khunnugiin ued kholbogdoh buleg  
 182 orshuulga. Mongolian Journal of Anthropology, Archaeology, and Ethnology, 3, 156–168; 11.  
 183 Excavated by D. Navaan in 2004. National University of Mongolia sample records; 12.  
 184 Excavated in 2006 by Z. Batsaikhan. National University of Mongolia sample records; 13.  
 185 Excavated by D. Navaan, M. Erdene, and C. Vanchigdash in 2008. National University of  
 186 Mongolia sample records; 14. Excavated by D. Navaan in 1980. National University of  
 187 Mongolia sample records; 15. Excavated in 2010 during Umengobi Expedition. National  
 188 University of Mongolia sample records; 16. Excavated by the NUM School of Anthropology and  
 189 Archaeology Summer Practice. National University of Mongolia sample records; 17. Excavated  
 190 nu Z. Batisaikhan, and S. Ulzibayar AA course II, 2005. National University of Mongolia sample  
 191 records; 18. Excavated 2010, Umungobi, South Gobi. National University of Mongolia sample  
 192 records; 19. Excavated by NUM Anthrpology and Archaeology II and III Summer Practice.  
 193 National University of Mongolia sample records; 20. Excavated in 1979, at Kharkhorin, by Ser-  
 194 Odjab, Bayar, and Tumen. National University of Mongolia sample records; 21. Excavated by C.  
 195 Ulziibayar in 2009 on the Northern Mongolian Expedition.  
 196

Archaeological Culture	Archaeological Site	Individual ID	Calibrated Radiocarbon Date BCE	Species/Taxonomic Group
<b>Early Bronze</b>				
Afanasievo	Shatar Chuluu <sup>1</sup>	AT-26	3316-2918	Bovinae/Ovis*
Chemurchek	Khundii Gobi <sup>2</sup>	AT-628	3310-2919	Ovis, Bovinae/Ovis
Chemurchek/ Afanasievo	Khuurai Gobi <sup>2</sup>	AT-635	2618-2487	None Detected
Chemurchek	Yagshiin Huduu <sup>2</sup>	AT-590b	2567-2468	Ovis, Bovinae/Ovis*, Caprinae
<b>Middle Bronze</b>				
Ulaanzuukh	Ulaanzuukh <sup>3</sup>	AT-823	1391-1209	None Detected
		AT-921	1412-1266	Capra, Ovis, Bovidae
		AT-923	No Date	Bovinae/Ovis*
		AT-824	1402-1279	None Detected
		AT-769A	1608-1416	Bovidae, Bovinae/Ovis*
		AT-769B	1509-1439	None Detected
<b>Late Bronze</b>				
Baitag	Uliastai River, Central Terrace <sup>4</sup>	AT-676	1277-1057	Equus, Capra, Ovis, Bovinae
Deer Stone/ Khirigsuur	Berkh Uul <sup>5</sup>	AT-905	1371-1121	Ovis, Capra, Bovinae/Ovis*
Slab Burial	Shunkhlai Uul <sup>6</sup>	AT-233	1072-903	Equus, Capra, Ovis, Bos
Undefined	Khoit Tsenkher <sup>7</sup>	AT-398	1056-904	Capra,

				Bovinae/ <i>Ovis</i> *
<b>Early Iron</b>				
Chandmani	Chandman Uul	AT-56	971-843	<i>Equus, Capra, Ovis, Bovinae</i>
		AT-121	358-195	None Detected
Slab Grave	Dartsagt <sup>9</sup>	AT-766	750-407	None Detected
<b>Late Iron</b>				
Xiongnu	Tamiryn Ulaan Khoshuu <sup>10</sup>	AT-728	No Date	<i>Equus</i>
Xiongnu	Duulaga Uul <sup>11</sup>	AT-605	43 BCE - 51 AD	<i>Bos, Bovinae/Ovis*, Caprinae</i>
Iron Age	Ulaanzuukh	AT-885	96-61 cal AD	<i>Caprinae, Bovinae/Ovis*</i>
<b>Early Medieval</b>				
Turkic	Uliastain dood denj	AT-675	650-765 cal AD	None Detected
<b>Late Medieval (Mongol Period)</b>				
Mongol Empire	Sharga Uul <sup>12</sup>	AT-701	No Date	<i>Equus</i>
Mongol Empire	Del Khad <sup>13</sup>	AT-775	No Date	<i>Equus, Ovis</i>
Mongol Empire	Zaraa Tolgoi <sup>14</sup>	AT-271	No Date	<i>Equus, Bovinae/Ovis*</i>
Mongol Empire	Banzart Khairkhan <sup>15</sup>	AT-846	No Date	<i>Equus, Bovinae/Ovis*</i>
Mongol Empire	Tahiltyn Khotgot <sup>16</sup>	AT-360	No Date	<i>Equus</i>
Mongol Empire	Burgaldain Khundii <sup>17</sup>	AT-650	No Date	<i>Equus, Caprinae, Camelus</i>
Mongol Empire	Ganzagad <sup>18</sup>	AT-835	No Date	<i>Equus, Capra, Bovinae/Ovis*</i>
Mongol Empire	Uguumur Tsuvaraa Uul <sup>19</sup>	AT-549	No Date	<i>Capra, Ovis, Bovinae</i>
Mongol Empire	Khoit Tsenkher, Tavgatain Am <sup>7</sup>	AT-354	1158-1252 AD	<i>Equus, Caprinae, Bovidae</i>
Mongol Empire	Kharkhorin <sup>20</sup>	AT-512	No Date	None Detected
Mongol Empire	Mori Baridag <sup>21</sup>	AT-800	No Date	None Detected

197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210

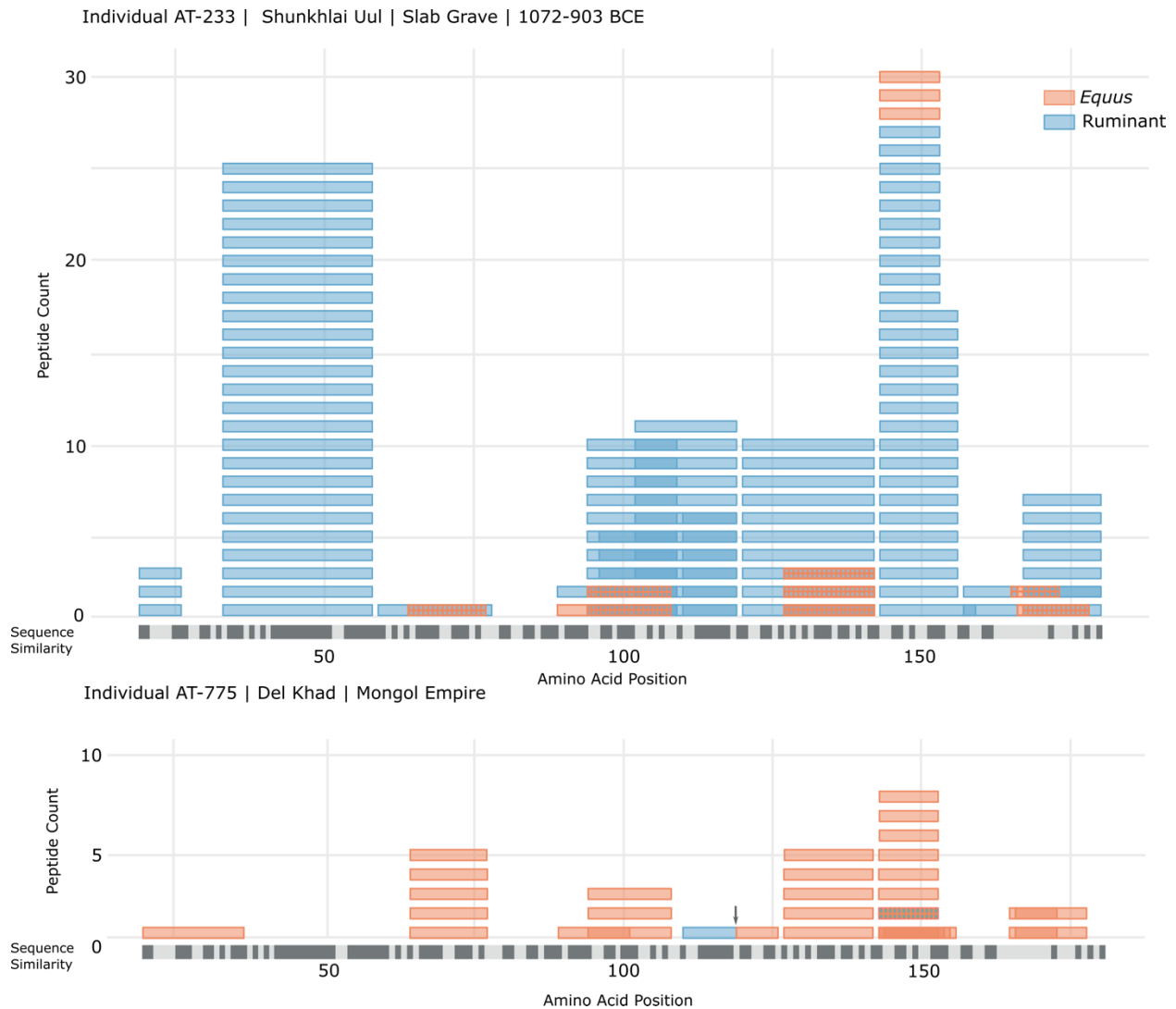
We find evidence of milk proteins in the earliest directly dated individual in our sample set, AT-26 (3316-2918 cal BCE; 2-sigma range) at the Afanasievo burials of Shatar Chuluu, the earliest known mounded burial features associated with pastoral economies in the territory of Mongolia. Specifically, we observe peptides deriving from a taxonomically ambiguous region of the milk whey protein BLG, where the species can be assigned as a bovid in bovine subfamily (cow, yak, bison, water buffalo) or *Ovis* genus (sheep), making a more specific taxonomic assignment for this individual's milk consumption more challenging. In the two individuals associated with Chermurchek culture at the sites of Khundii Gobi (2886-2577 cal BCE; 2-sigma range) and Yagshiin Khuduu (2567-2468 cal BCE; 2-sigma range), we detect milk peptides matching to the subfamily caprinae (sheep or goat) and the genus *Ovis* (sheep) from BLG and also alpha-S1-casein. At Khundii Gobi, these identifications are specific to sheep (with 6 BLG peptides identified) among others that are specific only to the subfamily caprinae and the infraorder



211 Pecora (all even-toed ruminant mammals). These results align with recent archaeofaunal data  
212 from the early 2nd millennia BCE, which suggest a significant role for sheep in the prehistoric  
213 economy of pastoral occupants of the Mongolian Altai<sup>28</sup>.

214  
215 In the Middle Bronze Age (*c.* 1800-1200 BCE) the consumption of ruminant dairy milk can be  
216 seen in four of seven individuals analyzed in the central eastern site of Ulaanzuukh, and in seven  
217 of nine individuals in the previously published Khövsgöl sites in northern central Mongolia.  
218 Ulaanzuukh individuals date to slightly earlier than the Bronze Age burial sites analyzed in  
219 Jeong et al <sup>29</sup>, where evidence of ruminant milk consumption was found in individuals associated  
220 with slope burials and khirigsuur ritual monuments. At Ulaanzuukh, BLG is the most frequently  
221 recovered protein across all samples, with peptides from alpha-S1-casein and kappa casein  
222 (proteins which are associated more with milk curds than milk whey) identified to a lesser extent.  
223 The dental calculus from the three Ulaanzuukh individuals who did not show evidence of dairy  
224 consumption exhibited a poor level of protein preservation, with a general absence of typical  
225 salivary and bacterial proteins reported previously in dental calculus studies<sup>29</sup>, which may suggest  
226 that an absence of evidence for milk consumption in these individuals could be due to overall  
227 poor biomolecular preservation.

228  
229 At Bronze Age sites dating to after 1200 BCE, we found evidence for dairy consumption in all  
230 four individuals tested. We identified ruminant milk peptides from the same two proteins (BLG,  
231 alpha-S1-casein) identified in the previous time period, as well as from two additional proteins:  
232 alpha lactalbumin and beta-casein. In addition to ruminant milk proteins, we also detected the  
233 first palaeoproteomic evidence of horse (*Equus*) milk, including horse-specific peptides from  
234 BLG I and II (horse BLG is derived from two paralogous genes) in 2 of 4 individuals. An  
235 individual from the site of Shunklai Uul (*c.* 1000 BCE), in central Mongolia, had 126 peptide  
236 spectral matches (PSMs) from ruminant BLG, another 50 from horse BLG I and II (Figure 3),  
237 and an additional nine from alpha-S1- and beta-casein and alpha-lactalbumin.



238  
239

240 **Figure 3. Alignment of observed BLG peptides for two individuals analysed in this study**  
 241 **(AT-233, Mid-Late Bronze Age; and AT-775, Mongolian Empire), showing the number of**  
 242 ***Equus* (orange) and ruminant (blue) BLG peptides detected.** For *Equus*, peptides from both  
 243 BLG1 and BLG2 paralogs are displayed (see SI Table 5 for data associated with figure). Where  
 244 peptides from these two taxa overlap this has been indicated by a blue/orange cross-hatch  
 245 pattern. The arrow in Individual AT-775 indicates two contiguous but independent peptides.  
 246 Beneath each individual is a consensus sequence of *Bos taurus* BLG (UniProt: P02754) and  
 247 *Equus caballus* BLG1 (UniProt: P02758) with dark grey indicating sequence identity, and pale  
 248 grey indicating sites with sequence differences.

249  
250

251 Of the three Early Iron Age individuals (800-400 BCE), two from the northwestern Mongolia  
 252 site of Chandman Mountain and another from Dartsagt (north-central Mongolia), all exhibited  
 253 moderate to high preservation and contained an abundance of human and oral microbiome  
 254 proteins. Two of these contained no robustly identified milk peptides, and in contrast one  
 255 individual from Chandman Mountain contained an abundance of PSMs to milk proteins. During

256 this period, BLG peptides specific to both sheep and goat were detected, along with others that  
257 can be assigned to the higher taxonomic orders of caprinae and bovidae. Peptides derived from  
258 casein and alpha-lactalbumin specific to caprines, and those from both BLG I and II specific to  
259 equine milk.

260  
261 In the Late Iron Age, during the time of the Xiongnu Empire (c. 200 BCE - 300 AD), calculus  
262 from each of the three individuals studied contained evidence for dairy consumption. One  
263 individual had PSMs specific to only horse BLG, and the second had *Bos* specific beta-  
264 lactoglobulin and caprine alpha-S1-casein peptides. The third individual had ruminant casein and  
265 whey proteins (BLG, alpha-S1-casein) as well as lysozyme C peptides specific to *Equus*. In the  
266 post-Xiongnu period, a single individual archaeologically classified as Turkic era did not display  
267 any evidence of milk consumption.

268  
269 Nine of the eleven Mongol Empire individuals displayed evidence for the consumption of dairy,  
270 with many individuals showing evidence for the consumption of multiple species. For example,  
271 one individual showed evidence for the consumption of ruminant, equine, and camel milk, whilst  
272 a further five individuals displayed evidence for the consumption of both ruminant and horse  
273 dairy products (Figure 3). During this period, we observed the first evidence for the consumption  
274 of camel milk, through the detection of peptidoglycan recognition protein 1 (UniProtKB:  
275 Q9GK12), an immune protein that has been isolated from modern camel milk, which increases  
276 dramatically during mastitis.

277  
278 Deamidation of glutamine and asparagine has been proposed as a marker of taphonomic  
279 degradation in ancient proteins<sup>30</sup>. We applied an analysis of bulk deamidation<sup>31</sup> to 5 individuals  
280 from this study to examine potential patterns of archaeological degradation in milk, which  
281 suggests that the milk proteins in our samples are ancient, as they appear to be of similar  
282 deamidation levels as the human oral proteins in the calculus. We observed that the older  
283 samples generally showed higher levels of deamidation. The early Bronze Age individuals  
284 displayed an average of 24.3% glutamine deamidation, the Late Bronze Age 18.4%, and the  
285 Mongol era individual 3.3%. The same pattern was observed in the deamidation of asparagine,  
286 with an average of 55.8% deamidation in the Early Bronze Age and 36.9% in the Late Bronze  
287 Age. However, the Mongol era individual does not fit this pattern in terms of asparagine  
288 deamidation, with 48.3% deamidation (Supplementary Table S1).

289  
290

## 291 **Discussion**

292

293 *Earliest evidence for dairying is associated with Western Steppe herder archaeological cultures*

294

295 Our results demonstrate the oldest known evidence of dairy consumption in Mongolia and the  
296 eastern Eurasian steppe (c. 3000 BCE - 2500 BCE), in the form of Early Bronze Age Afanasievo  
297 and Chemurchek-associated individuals in both central and Western Mongolia (Table 1).

298 Previous ancient DNA analysis of one of the individuals at Shatar Chuluu exhibiting ruminant  
299 dairy proteins (AT-26) was shown to have a non-local mitochondrial haplogroup consistent with  
300 WSH populations<sup>32</sup>, supporting the interpretation of individuals associated with the Afanasievo  
301 culture as migrants from eastern Europe via the Russian Altai<sup>33-35</sup> and a likely vector for the initial

302 introduction of domestic animals into Mongolia<sup>8</sup>. Our identification of ruminant milk proteins  
303 (*Ovis* and *Bovinae/Ovis*) support the domestic nature of fauna found in these burial features, and  
304 indicate that dairy pastoralism formed an important element of the subsistence base of these late  
305 4<sup>th</sup> millennium BCE transcontinental migrants. Most significantly, they suggest that human  
306 migrations associated with the expansion of the Afanasievo culture present a viable candidate for  
307 the initial introduction of dairy and domestic livestock into eastern Eurasia. In this study, the  
308 earliest individual in this dataset displayed evidence of dairy consumption. To track the first  
309 instance of dairy consumption in the Eastern Steppe, it would be necessary to analyse individuals  
310 from earlier time periods. This would be particularly informative to untangle the presence or  
311 absence of dairying prior to Yamnaya-Afanasievo migrations.

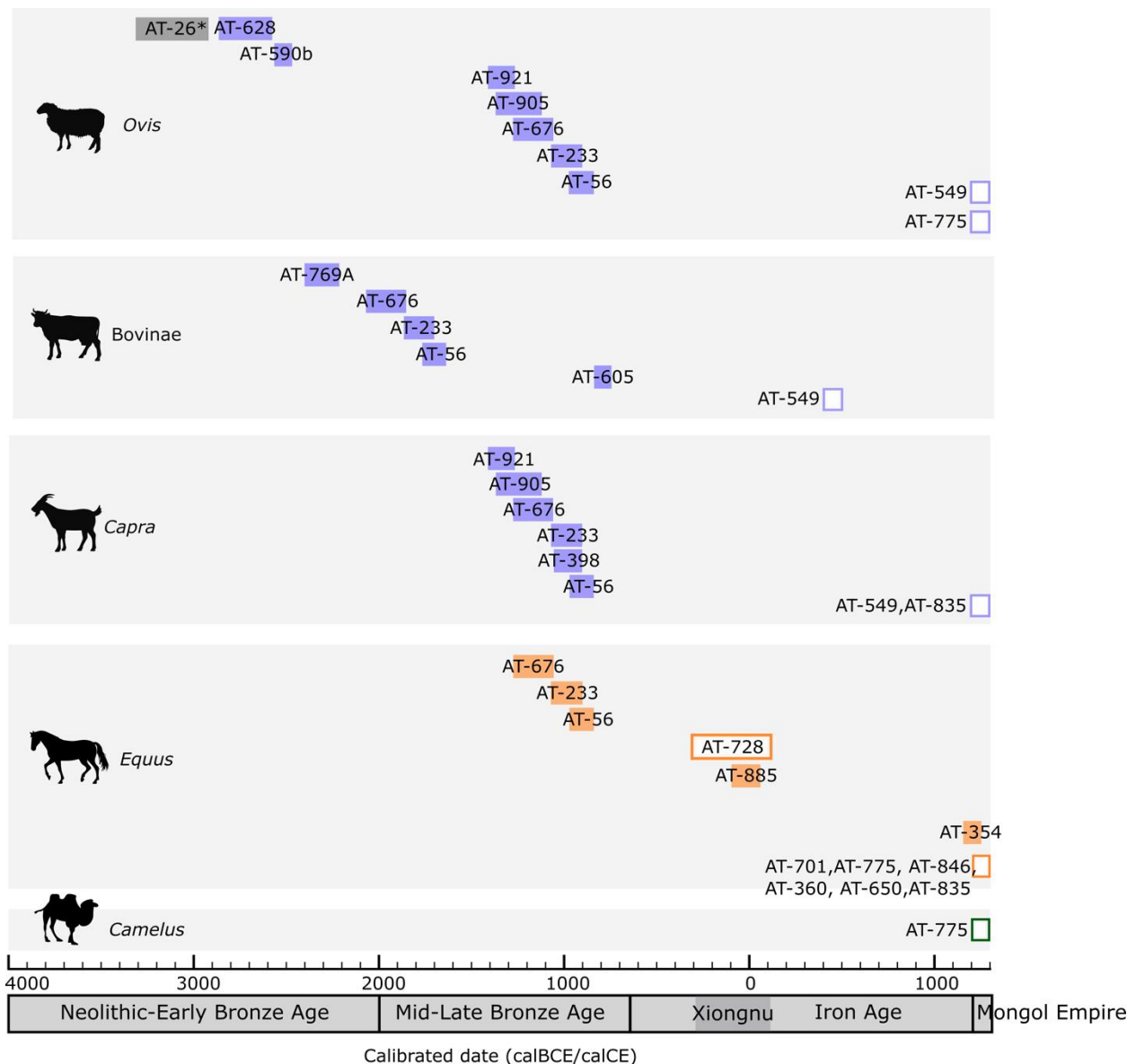
312

### 313 *The antiquity of eastern steppe horse milk consumption*

314

315 Temporal patterning in our protein results suggest that horse milk consumption played a key role  
316 in the emergence and proliferation of mobile pastoralism in Mongolia (Figure 4). Today, horses  
317 play a vital role in traditional Central Asian pastoral lifeways, improving herd management as  
318 well as providing a primary source of meat and milk. Horseback herders can manage larger herd  
319 sizes, and horses can break through snow and ice to access the sustenance underneath, exposing  
320 grass oases for other animals in the herd<sup>36-38</sup>. We observe direct evidence of horse milk  
321 consumption on the Eastern steppe, in the form of equine (*Equus ferus caballus*) specific  
322 peptides from the milk whey proteins BLG (I and II) and lysozyme C in individuals associated  
323 with the Baitag and Slab Grave cultures in western Mongolia dated to the late second millennium  
324 BCE (Table 1). In addition to the appearance of horses in dietary assemblages<sup>14</sup>, this time period  
325 is linked with the proliferation of horses in ritual sites, the first direct archaeological evidence for  
326 horse bridling and riding<sup>18,39</sup>, the first evidence for horse breeding and management<sup>15,16</sup>, innovations  
327 in horse healthcare<sup>17</sup>, an expanded use of dry intermontane grasslands<sup>16,17,40</sup> and the emergence of  
328 mobile, horse-facilitated pastoralism in eastern Eurasia. Our findings suggest that the  
329 incorporation of horses into existing ruminant dairy herds may have been closely linked to this  
330 multi-faceted economic transformation in the use of horses<sup>40</sup>.

331



332  
 333  
 334  
 335  
 336  
 337  
 338  
 339  
 340  
 341  
 342  
 343  
 344  
 345  
 346

**Figure 4. Timeline of evidence for the consumption of different livestock milk in prehistoric and historic Mongolia.** Radiocarbon dates for each individual were calibrated using OxCal (OxCal v4.3.2 Bronk Ramsey<sup>35</sup>; r:5 IntCal13 atmospheric curve<sup>36</sup>) and resulting radiocarbon probabilities were grouped by the taxa of dairy proteins identified in that individual (indicated by AT- numbers), with ruminant taxa (*Ovis*, *Capra* and *Bovinae*) indicated in purple, *Equus* indicated by orange and *Camelus* indicated by green. Dairy peptides identified in individual AT-26 (indicated with an asterisk) are specific to *Bovinae/Ovis*. Individuals without direct radiocarbon dates are indicated by unfilled boxes and are placed on the timeline based on the estimated time spans for the Xiongnu and Mongol Empires.

Following the Bronze Age, direct proteomic evidence for horse milk consumption continues through the imperial Xiongnu and Mongol periods (Table 1), in agreement with extensive textual and zooarchaeological evidence underscoring their significance to historic economies. During

347 the Iron Age, we identify horse milk proteins at Tamiryn Ulaan Khoshuu, a site within the  
348 heartland of the Xiongnu Empire<sup>43,44</sup>. Historical Chinese documents record that an assortment of  
349 dairy products were consumed during the Xiongnu period (c. 200 BCE - 100 AD), including  
350 dried curds from ruminant milk (*aaruul*), but *lao* “horse alcohol” was the most consistently  
351 referenced dairy product, and it held a prominent place in the cultural practices and identities of  
352 the steppe peoples<sup>45-47</sup>. By the Mongol period, horse milk consumption appears in among >80%  
353 (9 of 11) of tested individuals, a finding that matches historical accounts for widespread  
354 consumption of fermented mare’s milk, known as airag (Mongolian) or koumiss/kymyz (Turkic  
355 languages)<sup>20</sup>.

356  
357 Horse milk differs from ruminant milks in important ways, in particular, it contains less curd  
358 protein (caseins) and much more lactose<sup>36,48,49</sup>. While the lower casein content makes it undesirable  
359 for making most dried curd products, like *aaruul*, a staple of the traditional Mongolian diet, its  
360 high lactose content makes it highly suitable for making alcoholic beverages with ethanol  
361 contents as high as 10-12%<sup>30</sup>. Communal airag drinking was and continues to be an important  
362 social activity (Figure 1), and it has been frequently noted in both Mongol and foreign historical  
363 texts. The cultural significance of airag continued throughout the Mongol period, with social  
364 gatherings at the time referred to as “going to drink airag with people”, and social rankings  
365 reinforced by how close one sat in relation to the pitchers of airag. Favored associates were  
366 charged with serving the airag, and with choosing the order in which airag was served at feasts<sup>20</sup>.  
367 At the Mongol capital of Kharkorum, a silver fountain was said to flow with fermented mare’s  
368 milk, dispensing airag<sup>21</sup>.

369  
370

#### 371 *Identification of other milks and fermentation agents*

372

373 In addition to cattle, sheep, goats, and horses, domesticated Bactrian camels (*Camelus*  
374 *bactrianus*), yaks (*Bos grunniens*), and reindeer (*Rangifer tarandus*) are also milked in  
375 contemporary Mongolia; however, very little is known about the dairying history of these three  
376 species. Here we report protein evidence of camel milk consumption (together with horse and  
377 sheep milk) in a Mongol period individual buried in the Gobi Desert (Table 1), a habitat of  
378 Bactrian camels<sup>32</sup>. Although not unique to milk in other mammal species, the camel protein we  
379 identify here, peptidoglycan recognition protein 1 (*PRP1*), is an important component of camel  
380 milk whey<sup>53,54</sup>. Mongol era historical accounts, such as the twelfth-century Secret History of the  
381 Mongols<sup>20</sup>, and historic accounts from foreign travelers into the empire, such as Marco Polo<sup>21</sup>,  
382 include stories of camels used for subsistence (milk, meat, and blood) and transport of people  
383 and *gers*. While likely not the earliest instance of camel milk drinking, the *PRP1* protein data  
384 presented here are the first biomolecular evidence for its consumption. Camel dairy use is not  
385 well understood, and this finding provides a necessary insight into exploitation of this historic  
386 traction animal for milk.

387

388 We did not detect any milk peptides specifically identified as yak or reindeer in this study. While  
389 yak is a commonly herded species in Mongolia today, their past use as a dairying animal is not  
390 well understood. Yak proteins are difficult to specifically identify due to their sequence  
391 similarity to other cattle species. Yak beta-lactoglobulin, for example, differs from that of cattle  
392 at only a single amino acid across the entire BLG protein. While it is possible that some of the

393 peptides assigned in this study to *Bos* or the higher taxonomies of Pecora, Bovidae, and Bovinae  
394 could have originated from domestic yak, we only observed cattle-specific variants when  
395 sufficient protein coverage enabled it. The absence of reindeer-specific milk consumption may  
396 be expected based on historical accounts on the use of this animal for dairying in this region.  
397 Contemporary reindeer herders in Mongolia do milk their animals, but these families migrated  
398 from Siberia into northern Mongolia only in the last century <sup>55,56</sup>.

399  
400 Our results provide the earliest evidence for dairy pastoralism in Mongolia's first herding  
401 societies, pushing back previous estimates of dairying in the Eastern steppe by more than 1,700  
402 years, and tracing pastoralist connections between the Western and Eastern steppes to the Early  
403 Bronze Age. Our results show that within 5,000 years after the earliest evidence for dairying in  
404 the Near East, this practice and its associated animals had spread more than 7,000 thousand  
405 kilometers eastward to become a successful mode of subsistence on the Mongolian steppe. While  
406 the routes of this movement remain to be fully understood, our data suggest that dairy  
407 pastoralism, and in particular the emergence of horse riding and horse milking *c.* 1200 BCE,  
408 provided the economic, political, and social support necessary for the success of subsequent  
409 nomadic empires on the vast grasslands of Eurasia.

410  
411 In the present study, we did not identify any proteins specific to any bacterial taxa or other  
412 fermentation agents used in the processing of milk into other dairy products. Although  
413 processing agents were not found in the samples analysed in this study, it does not mean these  
414 were not used in ancient and historic Mongolia, and future studies should continue to look for  
415 their presence alongside milk peptides.

## 416 417 418 **Methods**

### 419 *Sample collection*

420 Dental calculus samples were collected from the Department of Archaeology at the National  
421 University of Mongolia (NUM) from 32 previously excavated individuals (Table 1; listed by  
422 NUM accession number). Individuals were selected from archaeological sites assigned to time  
423 periods between the Neolithic and the Mongol Period. Dental calculus was removed from the  
424 tooth using sterilized dental scalars and stored in Eppendorf tubes until extraction. Nitrile gloves  
425 were used during sample collection to avoid contamination from skin proteins. Samples were  
426 exported to the Max Planck Institute for the Science of Human History under permission from  
427 the Ministry of Culture, Education, Science and Sports (Export number 10/413 (7b/52) was  
428 received on 2nd February, 2017 #A0109258, MN DE 7 643). Protein extractions were conducted  
429 in a dedicated laboratory for the extraction of ancient proteins at the Max Planck Institute for the  
430 Science of Human History, Jena, using a Filter Aided Sample Preparation (FASP) protocol  
431 previously published in Jeong et al.<sup>22</sup>. Following protein extraction, digested peptides were stored  
432 at -80°C before being analyzed by LC-MS/MS at the Functional Genomics Center Zürich  
433 (FGCZ) at the ETH/University of Zürich, Switzerland.

### 434 435 *LC-MS/MS Analysis*

436 Mass spectrometry analysis was performed on a Q Exactive HF mass spectrometer (Thermo  
437 Scientific) equipped with a Digital PicoView source (New Objective) and coupled to a M-Class  
438 UPLC (Waters). Solvent composition at the two channels was 0.1% formic acid for channel A

439 and 0.1% formic acid, 99.9% acetonitrile for channel B. Column temperature was 50°C. For each  
440 sample 4 µL of peptides were loaded on a commercial ACQUITY UPLC M-Class Symmetry  
441 C18 Trap Column (100Å, 5 µm, 180 µm x 20 mm, Waters) followed by ACQUITY UPLC M-  
442 Class HSS T3 Column (100Å, 1.8 µm, 75 µm X 250 mm, Waters). The peptides were eluted at a  
443 flow rate of 300 nL/min by a gradient from 5 to 40% B in 120 min. Column was cleaned after  
444 the run by increasing to 98 % B and holding 98 % B for 5 min prior to re-establishing loading  
445 condition. Samples were acquired in a randomized order. The mass spectrometer was operated in  
446 data-dependent mode (DDA), acquiring a full-scan MS spectra (350–1,500 m/z) at a resolution  
447 of 120,000 at 200 m/z after accumulation to a target value of 3,000,000, and a maximum  
448 injection time of 50 ms followed by HCD (higher-energy collision dissociation) fragmentation  
449 on the twelve most intense signals per cycle. HCD spectra were acquired at a resolution of  
450 30,000 using a normalized collision energy of 28 and a maximum injection time of 50 ms. The  
451 automatic gain control (AGC) was set to 100,000 ions. Charge state screening was enabled.  
452 Singly, unassigned, and charge states higher than eight were rejected. Only precursors with  
453 intensity above 90,000 were selected for MS/MS. Precursor masses previously selected for  
454 MS/MS measurement were excluded from further selection for 30 s, and the exclusion window  
455 was set at 10 ppm. The samples were acquired using internal lock mass calibration on m/z  
456 371.1012 and 445.1200.

#### 457 458 *Data analysis*

459 In order to account for as much variation of milk-associated proteins as possible during MS/MS  
460 ion searches, a supplementary database of unreviewed milk protein sequences was curated from  
461 TrEMBL. As an additional source of dairy protein sequences, genomic data covering the 2 beta-  
462 lactoglobulin genes in ancient horses generated by Gaunitz and colleagues <sup>52</sup> were translated into  
463 amino acid sequences, aligned with their respective modern sequences, and any putatively  
464 divergent proteins were concatenated to the supplementary database. In total, 244 additional  
465 accessions from TrEMBL and 4 putatively divergent beta-lactoglobulin sequences from ancient  
466 horse genomes were added (Supplementary Table S1).

467  
468 Peak lists were generated from raw files by selecting the top 100 peaks using MSConvert from  
469 the ProteoWizard software package version 3.0.11781<sup>53</sup>. Each sample was searched using  
470 Mascot<sup>54</sup> (version 2.6.0) against Swiss-Prot in combination with the internally curated milk  
471 protein database (Supplementary Table S1). Results were exported from Mascot as csv files, and  
472 further processed through an internally created tool, MS-MARGE<sup>60</sup>  
473 (<https://bitbucket.org/rwhagan/ms-marge/src/master/>), to estimate the validity of peptide  
474 identifications and summarize the findings. MS-MARGE is an R script which relies on an  
475 Rmarkdown file to generate the following: an HTML report summarizing the search  
476 (Supplementary File 2), a csv file containing confidently identified PSMs, and a FASTA file of  
477 confidently identified peptide sequences. As input, MS-MARGE accepts a csv file exported from  
478 a Mascot MS/MS ion search against an amino acid database containing decoyed sequences with  
479 the Group Protein Families option turned off. Additionally, two parameters can be provided: an  
480 expect value (e-value) cutoff, and a minimum number of peptides to support an identification  
481 (default 0.01 and 2, respectively). In order to estimate FDR at the PSM and protein level, MS-  
482 MARGE counts the number of decoy hits after filtering for e-value and minimum peptide  
483 support, and divides this value by the number of target hits minus the number of decoys. The  
484 resulting value is multiplied by 100 to provide an estimate of FDR in percentage. We aimed for a



485 protein FDR of under 5%, and a peptide FDR of under 2% (Supplementary Table S7). A  
486 minimum of two individual PSMs were required for specific protein identifications, and only  
487 peptides with an E-value of below 0.01 were accepted. After filtering criteria was applied, we  
488 observed a range of variation in the numbers of proteins identified, with samples ranging from 2  
489 to 209 confidently identified protein families.

490  
491 Deamidation levels in five samples (AT-628, AT-590b, AT-26, AT-233 and AT-835) were  
492 calculated in order to assess authenticity using a previously published approach<sup>21</sup> (Supplementary  
493 Figure 2 and Supplementary Table 2). These samples were specifically chosen to verify the  
494 antiquity of the oldest samples in this study, as well as a more recent sample from the Mongol  
495 period for comparison of deamination patterns. The raw MS/MS files for each were run through  
496 MaxQuant<sup>21</sup> version 1.6.2.6a against the previously described milk database and against the  
497 human proteome. Settings included a semi-tryptic search strategy allowing for a maximum of  
498 two missed cleavages was utilized, and the score cutoff for modified and unmodified peptides  
499 was set to 60 with no correction for FDR. Carbamidomethyl (C) was added as a fixed  
500 modification, while variable modifications included: Oxidation (M), Acetyl (Protein N-term),  
501 Deamidation (NQ), Gln->pyro-Glu, Glu->pyro-Glu, Phospo (ST) and Hydroxyproline. The  
502 deamidation levels of all milk proteins were averaged per sample. All peptides, including their  
503 post-translational modifications, are reported in [Supplementary File Mongolia Dairy Peptides  
504 Per Individual].

#### 505 506 *Radiocarbon dating*

507  
508 A total of 26 bones and teeth from 24 individuals were radiocarbon dated at two research  
509 facilities, the Oxford Radiocarbon Accelerator Unit (ORAU, lab code OxA) and the Groningen  
510 Radiocarbon Laboratory (lab code GrM). The ORAU followed routine pretreatment and  
511 measurement procedures<sup>22</sup>. In brief, between 200-600mg of bone or dentine was drilled using a  
512 hand-held dentist drill, and collagen was extracted through a series of chemical steps that  
513 involved immersion in HCl, removal of humic acids using NaOH and removal of adsorbed CO<sub>2</sub>  
514 via a final HCl wash. Only 4 of the samples prepared at the ORAU (OxA-36230, -36231, -  
515 36232, -36233) underwent ultrafiltration using Vivaspin ultrafilters, due to initial indications of  
516 poor collagen perseveration. Extracted collagen was frozen overnight and was lyophilized.  
517 Between 2-5 mg of collagen was combusted in an elemental analyser (EA) and its C and N stable  
518 isotopes were measured at an IRMS instrument linked to the EA, before excess gas CO<sub>2</sub> was  
519 collected, graphitized and measured at an HVEE accelerator, alongside blanks and standards.  
520 These were used for contamination calculation and final correction of the data. For these samples  
521 collagen yields ranged greatly from 0.8% to 17.8%, C:N ratio of the extracted collagen fall  
522 within expected ranges (3.2-3.4) with the exception of OxA-36233 (C:N=3.6) and %C in the  
523 combusted collagen was between 37-46%. The measurements are reported in radiocarbon years  
524 before present (BP), where BP=AD 1950. Samples from Oxford were calibrated using OxCal  
525 v4.3.2<sup>21</sup> and IntCal13 atmospheric curve<sup>22</sup>.

526  
527 For radiocarbon dates processed at the Groningen Radiocarbon Laboratory, samples were  
528 decalcified over at least a 24-hour period using mild acid (HCl, 2-4% w/vol; RT) at the Center  
529 for Stable Isotope Research at the University of Groningen. For each sample still not fully  
530 decalcified, the solution was refreshed, removing and storing soft portions separately in

531 demineralised water until further preparation. Soft and pliable fragments were rinsed thoroughly  
532 with demineralised water. Extracts were then exposed to NaOH (1%, ~30 min) to eliminate  
533 humic acids, rinsed to neutrality and treated once more with acid (HCl, 4% w/vol, 15 min). The  
534 raw collagen fraction was denatured to gelatin in acidified demineralised water (pH 3) at 80 °C  
535 for 18 hours. Before drying, the dissolved gelatin was filtered through a 50 µm mesh to eliminate  
536 any remaining foreign particulates, and the crystalline collagen scraped from the glass.  
537 Approximately 4 mg aliquots of the reduced carbon fraction were then weighed into tin capsules  
538 for combustion in an Elemental Analyser (EA, IsotopeCube NCS, Elementar®). The EA was  
539 coupled to an Isotope Ratio Mass Spectrometer (IRMS, Isoprime® 100), allowing the d<sup>13</sup>C value  
540 of the sample to be measured, as well as a fully automated cryogenic system to trap CO<sub>2</sub> liberated  
541 on combustion. After run completion, the individual reaction vessels were transferred to a  
542 graphitisation manifold, where a stoichiometric excess of H<sub>2</sub> gas (1: 2.5) was added, and the CO<sub>2</sub>  
543 gas reduced to graphite over an Fe(s) catalyst. The graphite samples were then pressed, and the  
544 radioisotopic ratio determined on a MICADAS accelerator mass spectrometer. Samples from  
545 Groningen were calibrated using OxCal v4.3.2<sup>41</sup> and IntCal13 atmospheric curve<sup>42</sup>.

546

#### 547 **Data Availability**

548 Raw and processed MS/MS data from blanks, instrument washes and samples are available to  
549 download via the PRIDE partner repository under accession code PXD014730. MS-MARGE, an  
550 R script used to estimate the validity of peptide identifications and summarize the findings is  
551 available for use via Bitbucket: <https://bitbucket.org/rwhagan/ms-marge/src/master/>. The custom  
552 dairy database used to analyze the data in this study is available as a supplementary file (fasta  
553 format) in this study. The authors declare that all other data supporting the findings of this study  
554 are available within the paper and its supplementary information files.

555

#### 556 **References**

557

558

559. Hong, C. *et al.* Identification of milk component in ancient food residue by proteomics. *PLoS One* **7**,

560 [e37053](#) (2012).

561. Warinner, C. *et al.* Direct evidence of milk consumption from ancient human dental calculus. *Sci. Rep.* **4**,

562 [7104](#) (2014).

563. Hendy, J. *et al.* Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of

564 early farmers. *Nat. Commun.* **9**, 4064 (2018).

565. Greco, E. *et al.* Proteomic Analyses on an Ancient Egyptian Cheese and Biomolecular Evidence of

566 Brucellosis. *Anal. Chem.* **90**, 9673–9676 (2018).

567. Evershed, R. P. et al. Earliest date for milk use in the Near East and southeastern Europe linked to cattle  
568 herding. *Nature* **455**, 528–531 (2008).
569. Outram, A. K. et al. The earliest horse harnessing and milking. *Science* **323**, 1332–1335 (2009).
570. Wright, J. Households without Houses: Mobility and Moorings on the Eurasian Steppe. *J. Anthropol. Res.*  
571 **72**, 133–157 (2016).
572. Janz, L., Odsuren, D. & Bukhchuluun, D. Transitions in Palaeoecology and Technology: Hunter-  
573 Gatherers and Early Herders in the Gobi Desert. *Journal of World Prehistory* **30**, 1–80 (2017).
574. Kovalev, A. A., Erdenebaatar, D. & Rukavishnikova, I. V. A ritual complex with deer stones at Ushigiin  
575 Uvur, Mongolia: composition and construction stages. *Archaeology, Ethnology and Anthropology of*  
576 *Eurasia* **44**, 82–92 (2016).
577. Kovalev, A. A., Sergey, G. & Chuluunbat, M. *Earliest Europeans in the Heart of Asia: The Chemurchek*  
578 *(Qiemuerqieke) Cultural Phenomenon Vol. 2.* (Saint Petersburg State Museum - Institute of the Roerichs,  
579 2015).
580. Kovalev, A. *Earliest Europeans in the heart of Asia :The Chemurchek (Qiemuerqieke) cultural*  
581 *phenomenon Vol. 1.* (LEMA, 2014).
582. Kovalev, A. A. & Erdenebaatar, D. Discovery of new cultures of the Bronze Age in Mongolia according  
583 to the data obtained by the International Central Asian Archaeological Expedition. *Current*  
584 *archaeological research in Mongolia* 149–170 (2009).
585. Honeychurch, W. The Development of Cultural and Social Complexity in Mongolia. in *Handbook of East*  
586 *and Southeast Asian Archaeology* (eds. Habu, J., Lape, P. V. & Olsen, J. W.) 513–532 (Springer New  
587 York, 2017).
588. Houle, J.-L. Emergent Complexity on the Mongolian Steppe: Mobility, Territoriality, and the  
589 Development of Early Nomadic Polities. (University of Pittsburgh, 2010).
590. Allard, F., Erdenebaatar, D., Olsen, S., Cavalla, A. & Maggiore, E. Ritual and horses in Bronze Age and  
591 present-day Mongolia: Some preliminary observations from Khanuy Valley. *Social orders and social*  
592 *landscapes* 151–162 (2007).

5936. Taylor, W. Horse demography and use in Bronze Age Mongolia. *Quat. Int.* **436**, 270–282 (2017).
5947. Taylor, W. T. T. *et al.* Origins of equine dentistry. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E6707–E6715  
595 (2018).
5968. Taylor, W. T. T. & Tuvshinjargal, T. Horseback riding, asymmetry, and changes to the equine skull:  
597 Evidence for mounted riding in Mongolia’s Late Bronze Age. *Care or Neglect* 134–154 (2018).
5989. Sima, Q. *Records of the grand historian: Han dynasty.* **65**, (Columbia University Press, 1993).
5990. de Rachewiltz, I. The Secret History of the Mongols: A Mongolian Epic Chronicle of the Thirteenth  
600 Century. (2015).
6021. Smith, J. M., Jr. Dietary Decadence and Dynastic Decline in the Mongol Empire. *J. South Asian Nat.*  
602 *Hist.* **34**, (2000).
6032. Jeong, C. *et al.* Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian  
604 steppe. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E11248–E11255 (2018).
6053. Mays, S. *et al.* Lives before and after Stonehenge: An osteobiographical study of four prehistoric burials  
606 recently excavated from the Stonehenge World Heritage Site. *Journal of Archaeological Science: Reports*  
607 **20**, 692–710 (2018).
6084. Charlton, S. *et al.* New insights into Neolithic milk consumption through proteomic analysis of dental  
609 calculus. *Archaeol. Anthropol. Sci.* (2019). doi:10.1007/s12520-019-00911-7
6105. Hendy, J. *et al.* Proteomic evidence of dietary sources in ancient dental calculus. *Proc. Biol. Sci.* **285**,  
611 (2018).
6126. Tumen, D., Khatanbaatar, D. & Erdene, M. Bronze Age graves in the Delgerkhaan mountain area of  
613 eastern Mongolia and the Ulaanzuukh culture. *Asian archaeology* **2**, 40–49 (2013).
6147. Eregzen, G. Ancient funeral monuments of Mongolia. *Mongolian National Academy of Sciences* (2016).
6158. Houle, J.-L. *Bronze Age Mongolia.* (Oxford University Press, 2016).
6169. Warinner, C. *et al.* Pathogens and host immunity in the ancient human oral cavity. *Nat. Genet.* **46**, 336–  
617 344 (2014).

6180. Schroeter, E. R. & Cleland, T. P. Glutamine deamidation: an indicator of antiquity, or preservational  
619 quality? *Rapid Commun. Mass Spectrom.* **30**, 251–255 (2016).
6201. Mackie, M. *et al.* Palaeoproteomic Profiling of Conservation Layers on a 14th Century Italian Wall  
621 Painting. *Angew. Chem. Int. Ed Engl.* (2018). doi:10.1002/anie.201713020
6222. Rogers, L. L. Understanding ancient human population genetics of the eastern Eurasian steppe through  
623 mitochondrial DNA analysis: Central Mongolian samples from the Neolithic, Bronze Age, Iron Age and  
624 Mongol Empire periods. (Indiana University, 2016).
6253. Allentoft, M. E. *et al.* Population genomics of Bronze Age Eurasia. *Nature* **522**, 167–172 (2015).
6264. Damgaard, P. de B. *et al.* 137 ancient human genomes from across the Eurasian steppes. *Nature* **557**,  
627 369–374 (2018).
6285. Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503  
629 (2015).
6306. Malacarne, M., Martuzzi, F., Summer, A. & Mariani, P. Protein and fat composition of mare's milk: some  
631 nutritional remarks with reference to human and cow's milk. *International Dairy Journal* **12**, 869–877  
632 (2002).
6337. Barłowska, J., Szwajkowska, M., Litwińczuk, Z. & Król, J. Nutritional Value and Technological  
634 Suitability of Milk from Various Animal Species Used for Dairy Production. *Compr. Rev. Food Sci. Food*  
635 *Saf.* **10**, 291–302 (2011).
6368. Buell, P. D. Steppe Foodways and History. *Asian Med.* **2**, 171–203 (2006).
6379. Taylor, W. T. T., Bayarsaikhan, J. & Tuvshinjargal, T. Equine cranial morphology and the identification  
638 of riding and chariotry in late Bronze Age Mongolia. *Antiquity* **89**, 854–871 (2015).
6390. Taylor, W. T. T. *et al.* A Bayesian chronology for early domestic horse use in the Eastern Steppe. *J.*  
640 *Archaeol. Sci.* **81**, 49–58 (2017/5).
6411. Ramsey, C. B. Methods for Summarizing Radiocarbon Datasets. *Radiocarbon* **59**, 1809–1833 (2017).
6422. Reimer, P. J. *et al.* IntCal13 and Marine13 Radiocarbon Age Calibration Curves 0–50,000 Years cal BP.  
643 *Radiocarbon* **55**, 1869–1887 (2013).

6443. Brosseder, U. & Miller, B. K. Xiongnu Archaeology. *Multidisciplinary Perspectives of the First Steppe*  
645 *Empire in Inner Asia. Bonn Contributions to Asian Archaeology* 5, (2011).
6444. Miller, B. K. Power Politics in the Xiongnu Empire. (University of Pennsylvania, 2009).
6475. Kuan, H. Huan Kuan 桓寬 (1st BCE). in *Discourse on Salt and Iron: Commentaries* (ed. 王利器 W. L.-  
648 C.) (1958).
6496. Ban, G. *Han shu*. 5, (Ding wen shu ju, 1962).
6507. Sima, Q. Shiji (Records of the grand historian). *Qin Dynasty Volume (transl. Wtson, B.) (Columbia*  
651 *University Press, New York, 1961)* (1959).
6528. Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I. & Chilliard, Y. Composition of goat and  
653 sheep milk products: An update. *Small Rumin. Res.* 79, 57–72 (2008).
6549. Salimei, E. & Fantuz, F. Equid milk for human consumption. *Int. Dairy J.* 24, 130–142 (2012).
6550. Badarch, D. & Zilinskas, R. A. *Mongolia Today: Science, Culture, Environment and Development.*  
656 (Routledge, 2015).
6571. (of Rubruck.), W. *The Mission of Friar William of Rubruck: His Journey to the Court of the Great Khan*  
658 *Mongke, 1253-1255.* (Hakluyt Society, 1990).
6592. Reading, R. P., Mix, H., Lhagvasuren, B. & Blumer, E. S. Status of wild Bactrian camels and other large  
660 ungulates in south-western Mongolia. *Oryx* 33, 247–255 (1999).
6653. Hailu, Y. *et al.* Functional and technological properties of camel milk proteins: a review. *J. Dairy Res.* 83,  
662 422–429 (2016).
6634. Kappeler, S. R., Heuberger, C., Farah, Z. & Puhan, Z. Expression of the peptidoglycan recognition  
664 protein, PGRP, in the lactating mammary gland. *J. Dairy Sci.* 87, 2660–2668 (2004).
6655. Keay, M. G. The Tsaatan Reindeer Herders of Mongolia : Forgotten lessons of of human-animal systems.  
666 *Encyclopedia of Animals and Humans* 1–4 (2006).
6676. Inamura, T. The transformation of the community of Tsaatan reindeer herders in Mongolia and their  
668 relationships with the outside world. *Senri Ethnol. Stud.* (2005).

6657. Gaunitz, C. et al. Ancient genomes revisit the ancestry of domestic and Przewalski's horses. *Science* **360**, 111–114 (2018).
6758. Chambers, M. C. et al. A cross-platform toolkit for mass spectrometry and proteomics. *Nat. Biotechnol.* **30**, 918–920 (2012).
6739. Perkins, D. N., Pappin, D. J., Creasy, D. M. & Cottrell, J. S. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**, 3551–3567 (1999).
6750. Hagan, R. *Mpi-Shh-Mascot Report GGenerator*. (2018).
6761. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized ppb-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26**, 1367 (2008).
6782. Brock, F., Higham, T., Ditchfield, P. & Ramsey, C. B. Current Pretreatment Methods for AMS Radiocarbon Dating at the Oxford Radiocarbon Accelerator Unit (Orau). *Radiocarbon* **52**, 103–112 (2010).

681

## 682 **Acknowledgements**

683

684 We thank the National Museum of Mongolia and the Ministry of Education, Culture, Science,  
685 and Sports for facilitating this research. We thank the Functional Genomics Center Zürich, Laura  
686 Kunz and Claudia Fortes for mass spectrometry analysis and helpful comments and suggestions  
687 for this study. We thank Dalaimyagmar, Bayandalai and Sukhbat-Nayamsuren from Khovsgol  
688 aimag for sharing their knowledge and insights into traditional Mongolian dairying practices,  
689 traditions, and herding strategies, and we thank Björn Reichhardt and Matthäus Rest for use of  
690 the photographs displayed in Figure 1. We thank Katerina Douka from the Oxford Radiocarbon  
691 Accelerator Unit, and Mike Dee from the Groningen Radiocarbon Laboratory for radiocarbon  
692 analysis. This research was supported by the Max Planck Society (S.W., A.V.M, W.T., R.H.,  
693 M.B., A.S., N.B., C.W. and J.H.), a Max Planck Society Donation Award (J.H. and C.W.), the  
694 U.S. National Science Foundation (BCS-1523264 to C.W.), and the European Research Council  
695 under the European Union's Horizon 2020 research and innovation programme under grant  
696 agreement number 804884-DAIRYCULTURES (C.W.). The protein database used for this  
697 research was produced within the framework of 'FoodTransforms: transformations of food in the  
698 Eastern Mediterranean Late Bronze Age' (ERC-2015-StG 678901-FoodTransforms) funded by  
699 the European Research Council. Deamidation analysis was funded by the Arts and Humanities  
700 Research Council (grant number AH/N005015/1).

701

## 702 **Author contributions**

703

704 S.W., W.T., N.B., C.W. and J.H. designed the research plan; S.W., M.B., S.G., M.H., E.M., and  
705 J.H. assessed archaeological collections, performed osteological assessments and subsampling.  
706 S.W., R.H., A.R., C.T., P.N., and J.H. performed laboratory work, mass spectrometry work and  
707 data analysis; R.H., A.S., A.R., C.T., J.G., P.S, and C.W. contributed to the development of data  
708 analysis tools; S.W., A.V.M., W.T., B.K.M., L.O., E.M., C.W., and J.H. and contributed to  
709 archaeological data interpretation. J.H. and S.W. generated the figures. S.W. and J.H wrote the  
710 paper with input from W.T., L.O. and C.W, and final approval from all authors.