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Teat morphology across five buffalo breeds: a multi-country collaborative study

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(Article begins on next page)

1 **Teat morphology across five buffalo breeds: a multy-country collaborative study**

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28 **a) Abstract**

29 The buffalo (*Bubalus bubalis*) is a species of worldwide importance, raised to produce milk, meat, and hides,
30 and often used as a working animal in rural contexts with low access to hi-tech solutions. In the present study
31 100 lactating buffaloes (50 primiparous and 50 pluriparous) of five popular breeds were recruited to
32 characterize and compare teat morphology. In particular, the focus was put on the Nili Ravi, Mediterranean,
33 Egyptian, Bulgarian Murrah and Azeri buffaloes raised in Pakistan, Italy, Egypt, Bulgaria and Iran,
34 respectively. In all countries, a longitudinal cross-section ultrasound was obtained before the milking to
35 measure teat parameters at individual level: overall, teat canal length (TCL) averaged 24.13 mm, teat diameter
36 (TD) 30.46 mm, cisternal diameter (CD) 17.80 mm and teat wall (TW) 7.12 mm. The most variable trait across
37 breeds was TCL which was positively correlated with CD and TD and negatively with TW, regardless of the
38 teat position (front/rear or left/right). A strong negative correlation was found between TW and CD (-0.43).
39 The analysis of variance revealed that the fixed effect of breed significantly affected all the traits except TD.
40 In fact, Bulgarian Murrah, Azeri, and Egyptian buffaloes presented the greatest estimate of TCL, whereas NR
41 the smallest (14.70 mm). The TW was maximum in Nili Ravi, Egyptian, and Mediterranean buffaloes, with
42 estimates equal to 8.19, 7.59, and 8.74 mm, respectively. Nili Ravi also showed the greatest TL (82.39 mm).
43 In terms of CD, the lowest least square mean was that of Mediterranean buffaloes (12.14 mm). Primiparous
44 and pluriparous buffaloes differed in terms of TD, TW and TL, with older animals presenting the highest least
45 square mean. In terms of position, instead, significant differences were observed for TD, CD and TL when
46 comparing front and rear teats, as left and right teats did not differ. Teat anatomy includes a set of heritable
47 morphological features and is therefore breed-dependent. Differences presented in this study could be
48 attributed to the divergent breeding objective and selective pressure across the five breeds; e.g., in some cases
49 such as Mediterranean buffalo, selection for decades was oriented to improve milk production and milkability
50 and achieve optimal conformation for mechanical milking. A better understanding of the mammary gland
51 anatomical descriptors can be informative of the history of a breed and could provide useful insights to guide
52 possible selection.

53 **Keywords:** genetic diversity, dairy buffalo, teat anatomy, mammary gland morphology.

54

55 **b) Introduction**

56 The water buffalo (*Bubalus bubalis*) is a widespread bovine specie, with a global population estimated at over
57 204 million heads. The 98% of animals are found in Asia, 0.8% in Africa, mainly Egypt, 0.9% in South
58 America, and 0.2% in Europe, mainly Italy (Minervino et al. 2020). Other than delivering products like milk,
59 meat, and hides, buffaloes have been used for working purpose in some confined/rural contexts with scarce
60 access to developed solutions.

61 Buffalo milk has a high nutritional value; in fact it has about twice the energy content of cow's milk, is
62 extremely rich in calcium and is a good source of essential minerals, amino acids and vitamins (Vargas-
63 Ramella et al. 2021; El Sabry et al. 2022). Water buffalo is classified into two types with different chromosome
64 number, i.e. the river (2n=50) and the swamp buffalo (2n=48). The river type makes up about 70% of the
65 world's water buffalo population and its milk accounts for a substantial share of total milk production in India
66 and Pakistan. Swamp buffaloes, on the other hand, are morphologically different from the former, being less
67 heavy and less productive in terms of milk (Minervino et al. 2020).

68 The domestication of the river buffalo likely occurred 6,300 years ago in north-western India, from where the
69 domesticated animals migrated westwards through south-western Asia, Egypt and Anatolia, reaching the
70 Balkans and the Italian peninsula. According to literature, there are 123 breeds, 90 just in Asia, many of which
71 are local and with a limited diffusion (Minervino et al. 2020). Out of the river buffalo breeds farmed globally,
72 the Murrah is the most popular as it has been intensively selected for milk production in the north-western
73 territories of India. Murrah buffaloes' main morphological characteristic is the presence of curled horns
74 (Minervino et al. 2020). In Bulgaria, the Bulgarian Murrah (BM) has been officially recognized as a population
75 and it is a crossbreed between the Indian Murrah and the Mediterranean breed. The second most represented
76 breed in the world is the Nili Ravi (NR), the most important breed in countries of South Asia like Pakistan and
77 India. As an example, the Indian Punjab region hosts over 10,000,000 NR heads in. The Azeri (AZ) – also
78 known as Caucasian - buffalo breed originates from the Indus Valley and descended from the Indian buffalo
79 populations (Minervino et al. 2020). The AZ can be found in Iran, Azerbaijan and along the Caspian Sea, but
80 can be found also in Georgia and Armenia.

81 The Egyptian breed (EG) was introduced into Egypt from India probably around the 7th century b.C. Animals
82 belonging to the EG breed are pivotal for the Egyptian dairy industry, as they produces 45% of the milk

83 consumed in the country. The Mediterranean (MI) is an Italian breed that descends from the river buffalo
84 introduced to Europe from India during the 8th century during the Arab occupation of Sicily and territories of
85 the southern Italian peninsula. Nowadays, the MI is officially recognized as a specialized dairy breed. Animals
86 in Italy are subjected to official genetic selection towards dairy purposes through the National Association of
87 Buffalo Species Breeders (ANASB). Most of the typical products obtained from MI milk are labelled as
88 Protected Designation of Origin (PDO), e.g. “Mozzarella di Bufala Campana” and “Ricotta di Bufala
89 Campana” (Italian Ministry of Agriculture & Forestry. Disciplinary of Production of Mozzarella di Bufala
90 Campana DOP, 11 February 2008).

91 In developed countries, particularly in Europe, the milking is becoming more and more mechanical rather than
92 manual. This happens for two main reasons: on one hand, to reduce tiring and expensive labor, and, on the
93 other hand, to improve hygienic conditions, udder health and quality of milk (Thomas, 2008).

94 The relationships between udder morphology, teat anatomy, milk production, milkability and udder health
95 have been extensively documented in the literature (Ambord et al. 2009, 2010; Boselli et al. 2014, 2020; Kaur
96 et al. 2018). Mammary gland conformation and type traits are often considered as selection criteria in breeding
97 programs of various dairy species (Borghese et al. 2007; Mirza et al. 2018). Recent research has shown that
98 the teat anatomy is typical of each buffalo breed, but can be affected by other factors, like husbandry system,
99 and milking type and routine (Boselli et al. 2014, 2020; Borghese et al. 2007; Klein et al. 2005; Costa et al.
100 2020).

101 The anatomical characteristics of the teat, besides being typical for each population, can also differ between
102 specialized (intensively selected for dairy) and not specialized breeds. A better understanding of the differences
103 between breeds is fundamental, as it allow customization of milking devices and procedures. As an example,
104 the teat cup liners and the working vacuum level of milking machine could be adjusted to perform the most
105 appropriate and less stressful milking procedures (Caria et al. 2012, 2013).

106 The aim of this study was to survey and compare external and internal teat measurements of five buffalo breeds
107 involving animals in early lactation. Teat canal length (TCL), teat length (TL), teat diameter (TD), cisternal
108 diameter (CD), and teat wall (TW) were measured in each teat before milking, covering possible variability
109 associated to effects like parity and teat position.

110

111 **c) Materials and Methods**

112 **Design of the Study**

113 This study was carried out between 2021 and 2022 in commercial or experimental pilot farms located in
114 Asia (n = 2), Europe (n = 2) and Africa (n = 1). One farm per country, i.e. per breed, was involved in order to
115 obtain data from MI, BM, NR, EG, and AZ buffaloes reared in Italy, Bulgaria, Pakistan, Egypt and Iran,
116 respectively. In each farm, 10 primiparous and 10 pluriparous clinically healthy lactating buffaloes in the first
117 month of lactation were randomly selected for the teats measurements collection. In total, 100 animals all with
118 4 functional teats were enrolled for this study.

119 The same protocol, preliminarily discussed, was adopted in each country. Briefly, in each farm only 1
120 operator (veterinarian) was trained and allowed to measure teats parameters on the animals before the evening
121 milking session. Measurements of primiparous and pluriparous took place in 2 different dates.

122
123 **Farms Involved**

124 The present section contains description of the five farms' main characteristics.

125 • **Italy:** commercial farm located in the Lazio region, where the herd size was 110 MI lactating buffaloes.
126 Animals were milked in a herringbone milk plant, with vacuum level of 42 kPa, 60 cycles per min, and
127 pulsator ratio at 60:40. Mechanical milking was performed twice a day – approximately at 4 a.m. and 4
128 p.m. Buffaloes were fed the same total mixed ration made using sorghum silage, alfalfa hay, and a special
129 mix formulated for a milk yield (MY) of 10.0 kg/d.

130 • **Bulgaria:** experimental farm of the Agricultural Institute – Shumen, located in the North-East of the
131 country. The farm hosted 68 lactating BM animals, which were machine milked in cans at a vacuum level
132 of 44 kPa, 60 cycles per min, and pulsator ratio 60:40. Mechanical milking was performed twice a day –
133 approximately at 4 a.m. and 4 p.m. The lactating group's diet involved per each buffalo 20 kg maize
134 silage, 2 kg leguminous hay, 4 kg cereal straw, and 4 kg compound feed. The ration was formulated for a
135 MY of 9.0 kg/d.

136 • **Pakistan:** NR herd of the Livestock Experiment Station of Buffalo Research Institute (Pattoki,
137 Pakistan). The farm had approximately 100 lactating buffaloes during the experimental period and
138 milking was performed manually and twice a day, approximately at 5 a.m. and 4 p.m. The daily ration

139 for lactating buffaloes consisted of seasonal green fodders (maize, sorghum, oats, and berseem) at a
140 rate of 10% of the body weight per animal, along with 4 kg of concentrate. The ration was formulated
141 for a MY of 10.0 kg/d.

- 142 • **Egypt:** the study was carried out at the Mehallet-Mousa Experimental Station located in the North
143 Nile Delta. The farm belongs to the Animal Production Research Institute (APRI, Kafr El-Sheikh
144 Governorate) and account for approximately 90 lactating buffaloes. Buffaloes were manually milked
145 twice a day at 6 a.m. and 4 p.m. throughout the whole lactation and were fed the same total mixed
146 ration: cover, corn silage, rice straw and concentrate, and mix for lactating cow. The ration was design
147 to cover the nutritional requirements of a lactating buffalo with a MY of 9.0 kg/d.
- 148 • **Iran:** the AZ buffaloes investigated in this study belonged to the Nikookar buffalo farm located in
149 Gilan Province, in north Iran, along the Caspian Sea. The farm hosted 500 buffaloes under semi-
150 intensive system. Milking was mechanically performed twice a day (6 a.m. and 6 p.m.) under a vacuum
151 level of 38 kPa, 60 cycles per min, and pulsator ratio 60:40 (Nikookar et al., 2021). Animals received
152 mixture of self-produced feedstuff (mainly rice stem, rice bran and wheat bran, soy bean, barley and
153 corn) formulated to satisfy the nutritional requirements, assuming a MY of 8.0 kg/d.

154 155 **Measurement of Anatomical Traits**

156 In each farm, a trained veterinarian performed the echography to assess the teat morphological
157 measurements before milking, as per protocol. Each teat was put in a cup of hand-warm water, and the
158 ultrasound probe was applied to the outside of the cup by using ultrasound gel (Ambord et al. 2009, 2010;
159 Boselli et al. 2014; Ozenc et al. 2020; Weiss et al. 2004). Longitudinal cross-section b-mode ultrasound images
160 were obtained (Fig. 1) for each teat and were used to measure TCL and, at 4 cm above the teat tip, the TD, the
161 left and the right wall thickness, and the CD. The total wall thickness (TW) was calculated as the average of
162 the left and the right wall as in Fig. 1. Once available, the teats measurements of the 100 buffaloes were inserted
163 into a shared database for data elaboration.

164 The images were recorded with the following instruments:

- 165 • **Italy - MI:** echograph Honda HS 101V (Honda Electronics, Japan) equipped with a 5-MHz linear-
166 array transducer.

- 167 • **Bulgaria - BM:** SonoScape S2 Vet (SonoScape, China), equipped with a multi-frequency (5.0-10.0
168 MHz) linear-array probe applied in vertical position with frequency adjusted at 7.0 MHz.
- 169 • **Pakistan - NR:** ultrasonic diagnostic scanner with linear probe of 7.5 MHz (HS 1500V, Honda
170 Electronics, Toyohashi, Japan).
- 171 • **Egypt - EG:** ultrasonography performed using an ultrasound SonoScape A5 Vet (Sonoscape Co. LTD,
172 Shenzhen, China) supplied with a multifrequency linear transducer (3.0-8.0 MHz).
- 173 • **Iran - AZ:** portable ultrasound machine Draminiski (Model vet mini, ROA 7.0.728605) coupled with
174 ultrasound probe of 5 MHz frequency.

175

176 **Data Analysis**

177 The SPSS software (version 19) was used for data manipulation, editing, and analysis. Descriptive
178 statistics, including the coefficient of variation (CV, %) and the standard error of the mean (SEM), were
179 calculated. To evaluate association between the traits, Pearson's correlations and their significance were
180 assessed in SPSS and the Shapiro-Wilk normality test was used to evaluate the distribution of the data. For the
181 analysis of variance, the GLM procedure of SPSS was adopted. The model accounted for the fixed effect of
182 breed, parity, and teat position, and two interactions as described below:

$$183 \quad y_{ijkl} = \mu + P_i + B_j + T_k + V_l + (P \times B)_{ij} + (P \times T)_{ik} + (P \times V)_{il} + (B \times T)_{jk} + (B \times V)_{jl} + e_{ijkl},$$

184 where: y_{ijkl} is the vector of phenotypic observations for each morphological trait recorded, μ is the
185 intercept, P is the fixed effect of the i -th parity of the buffalo ($i = 1$ and >1), B is the fixed effect of the j -th
186 breed (5 levels: MI, NR, BM, EG, and AZ), T is the fixed effect of the k -th position I (front vs rear), V is the
187 fixed effect of the l -th position II (left vs right), $(P \times B)$ is the first-order interaction between parity and breed,
188 $(P \times T)$ is the first-order interaction between parity and teat position I, $(P \times V)$ is the first-order interaction
189 between parity and teat position II, $(B \times T)$ is the first-order interaction between breed and teat position I,
190 $(B \times V)$ is the first-order interaction between breed and teat position II, and e_{ijkl} is the random residual. A
191 multiple comparison of least square means (LSM) was performed using the Bonferroni's test considering as
192 significant P -values < 0.05 .

193

194 **d) Results**

195 **Descriptive Statistics and Correlations**

196 A total of 400 teats belonging to 100 buffaloes were measured in 5 countries on animals belonging to the
197 local breed farmed and used for dairy purpose. The MY, which was recorded at evening milking after teat
198 anatomical trait measurements, ranged from a minimum of 3.31 kg to 4.15 kg in NR and BM (data not shown).
199 Overall and breed-specific descriptive statistics of the teat morphological traits are shown in Table 1. The CV
200 ranged from 18.45 to 37.93 % for TD and TCL, respectively. This indicated that the trait with the largest
201 variability across all the breeds was TCL. Within breed, the standard deviation achieved also values greater
202 than 9, with 9.13 and 10.97 mm in AZ and EG. The lowest standard deviation of TCL was calculated in NR
203 (3.62 mm).

204 As regards the data distribution, data points were plotted and visually inspected before the normality test
205 (Fig. 2). The traits presented a normal distribution with the only exception of TD ($P = 0.341$; Fig. 2). Looking
206 at raw means (Table 1), it was evident how TCL was on average lower in NR than in AZ (15.36 vs 29.13 mm),
207 whereas TL showed an opposite trend (82.38 vs 54.87 mm). In general, BM showed the lowest average TW
208 and the highest average CD (Table 1).

209 Pearson's correlation coefficients of the anatomical features studied are reported in Table 2; the same were
210 calculated also for front and rear teats separately and for the right and left lateral position separately (Table 2).
211 In some cases, the correlation assessed on the front teats differed from that of rear teats. As an example, TCL
212 was significantly correlated with TD, TW, and CD in front teats, however in rear teats the TCL was not in
213 significant association with TD (-0.003). In front teats the correlation between TD and TW was significant,
214 unlike rear teats. The same can be said for the correlation between TW and TL which was significant in
215 posterior but not in anterior position. Overall, the strongest associations were similar in front and rear teats,
216 and were calculated between TD and CD and between TW and CD.

217 The correlations between left and right teats, mirror those observed for the front and rear comparison, with
218 minor exceptions. TCL correlates with CD, TW and TD on the left side, whereas on the right side the
219 correlation with TW is not significant. TL correlated significantly with TD on the left side, but with CD and
220 TW on the right side.

221

222 **Fixed Effects**

223 The analysis of variance revealed that only part of the fixed effects significantly influenced the teat traits
224 investigated and that none of the interactions was significant, except for that between breed and parity. The
225 LSM of breed, parity, and teat position I and II are shown in Table 4. The TCL differed among the breeds,
226 with AZ, BM and EG showing a significantly higher estimate compared to MI and NR. NR showed the lowest
227 TCL (14.70 mm), which was 15.49 mm far from the greatest LSM (Table 3). A clear difference between breed
228 is evident also from Fig. 3, where two sample images of NR and BM teats are shown. Out of all the anatomical
229 traits measured, only TD was not affected by breed. The LSM were close to each other and statistically similar,
230 falling within the range from 29.62 and 31.22 mm (Table 3). The cistern with the lowest diameter was found
231 in MI, followed by NR and EG whose LSM were similar, i.e. 15.93 and 15.84 mm, respectively. As regards
232 the TW, results indicated that thickness is a variable feature in buffalo (Table 3). In fact, MI, NR and EG
233 showed higher LSM compared to AZ (6.30 mm) and BM (4.77 mm). Considering the TL, the LSM belonged
234 – in descending order – to NR, EG, BM, MI and AZ (Table 3), but LSM of EG and BM were similar (67.85
235 vs 72.50 mm, respectively).

236 Parity affected the variability of 3 out of the 5 traits investigated. In fact, only TD, TW and TL were
237 significantly different in the two categories, with pluriparous buffaloes generally showing greater LSM than
238 primiparous. While position I affected TD, TL and CD (Table 3) - with the rear quarters presenting a greater
239 LSM than front ones - lateral position (position II) did not significantly affect the traits.

240 Regarding the interaction between breed and parity, the analysis revealed that TCL was greater in
241 pluriparous than primiparous of AZ and BM, while in EG, MI and NR there was an opposite trend (data not
242 shown). Both TD and TW were higher in pluriparous BM, EG and NR, while in MI and AZ breed the LSM of
243 primiparous was greater compared to that of pluriparous (data not shown). The highest CD was found in the
244 primiparous of BM, EG and MI and in the pluriparous of AZ and NR. Finally, TL of primiparous was superior
245 only in the case of EG buffaloes; in the other breeds, in fact, older cows always showed longer teats.

246 **e) Discussion**

247 ***Breed-related Differences***

248 Ultrasound examination on teat anatomy in dairy species is a powerful tool to characterize udder anatomy
249 in a given breed or population and therefore having reference values to identify potential anomalous teats
250 (Ambord et al. 2009, 2010; Boselli et al. 2014; Thomas et al., 2004; Weiss et al. 2004). The method used in
251 the present study was standardized across the five countries to allow for an undisturbed and fair comparison
252 of the breeds investigated. Results of this study should be considered as preliminary. Data, in fact, refer to only
253 five farms (one per breed), therefore findings should be interpreted with caution as it was not possible to
254 account for the effect of farm, which, however, should be investigated in further studies.

255 The teat canal is the only orifice between the internal milk secretory system of the mammary gland and
256 the external environment. The anatomical characteristics of the teat reported in literature are different, because
257 there is large variability due to breed. In India, for the Murrah breed, Thomas et al. (2004) reported an average
258 TCL of 3.1 cm, with average values of 3.7 ± 0.2 and 3.0 ± 0.1 cm for the hind and fore teats, respectively. In
259 that study, authors found that TCL was similar in animals at different parities and stages of lactation. To reduce
260 disturbances, however, the protocol adopted in the present study was intended to get rid of the potential effect
261 of lactation stage and only animals in the first month of lactation were selected.

262 Pushkin Raj (2010) measured teats on both healthy and mastitic animals of the same breed raised in
263 Chennai (India), reporting an average TCL of 17.7 (front teats) and 20.3 mm (rear teats). For the crossbred
264 Murrah and Mediterranean \times Murrah, Bittner et al. (2010) observed TCL values between 14.0 and 15.0 mm in
265 front and rear teats, respectively which is close to the TCL of NR (15.36 mm; Table 1). Average TCL (Table
266 1) was intermediate between the value of Thomas et al. (2004) and those of Bittner et al. (2018) and Pushkin
267 Raj (2010). The TW was scarcely variable in the study of Bittner et al. (2010), falling between 0.71 to 0.72
268 cm. In this study, TW had a CV of 32.16% and there were differences among the breeds. Considering the raw
269 means in Table 1, the lowest and greatest average were found in BM and MI, respectively. The average TD of
270 all breeds (Table 1) was lower than that reported by Pushkin Raj (2010), whose values fell between 36.4 and
271 37.0 mm. CD recorded in BM was lower than that detected by Pushkin Raj (2010) whose range was: 2.72 -
272 3.14 cm.

273 In some studies carried out on MI buffaloes, the TCL was measured before application of a pre-milking
274 treatment: not stimulation, pre-stimulation, or oxytocin injection. Observed values fell between 23.6 and 27.8
275 mm (Ambord et al. 2009; Boselli et al. 2010). In the same breed, studies have demonstrated that there is a

276 reduction in the TCL with a 3 min pre-stimulation, i.e. with a decrease ranging from 25.5 to 19.5 mm (Ambord
277 et al. 2010) and from 23.6 to 14.8 mm (Boselli et al. 2014).

278 Changes in other anatomical parameters have been observed before and after the treatments (no
279 stimulation, manual pre-stimulation, or oxytocin injection). For instance, studies reported a reduction in TW
280 (from 97 to 84 mm), and an increase in both CD (from 13.1 to 17.1 mm) and TD (from 32.7 to 32.9 mm;
281 Boselli et al. 2014). Similarly, Ambord et al. (2009, 2010) reported TW to reduce (approximately 1 cm) and
282 observed increased CD (from 39 to 89 mm) and TD (29.2 to 29.6 cm). For the Anatolian-Mediterranean type
283 breed, a recent study conducted by Ozenc et al. (2020), showed a large variability of teat traits in relation to
284 animal temperament (docile or nervous), pre-stimulation length (0, 3 and 6 min), and teat position. In absence
285 of stimulation, TCL of buffaloes fell between 18.5 to 23.5 mm; however, after a few min of pre-stimulation in
286 the same animals the TCL reduced up to 22.0 mm (Ozenc et al., 2020). El-Ghousien et al. (2022) reported an
287 average TCL of about 13.0 mm in EG buffaloes, which is very low compared to the present study (Table 1).
288 Nevertheless, it should be pointed out that El-Ghousien et al. (2022) measured the teats *post-mortem* and with
289 different instrumental methods compared to the present study. The same authors reported TD in EG buffaloes
290 to vary from 22.0 to 23.0 mm, while in this study TD averaged 31.01 mm in the same breed. Al-Galil ASA
291 (2016) for the same breed, found the normal teat walls appeared as high reflective structure with three discrete
292 layers; the outer hyperechoic layer, the middle thicker hypoechoic layer and the inner hyperechoic layer. The
293 teat cistern appeared as a dilated anechoic area with few hypoechogenic dots corresponding to the milk
294 contents. In subclinical mastitis, the teat canal and cistern appeared with irregular contour lining, homogenous
295 hypoechogenic contents, narrower lumen, slightly thickened wall and loss the characteristic three layered
296 appearances. In this way the authors measured teat TW thickness from 0.63 to 1.05 cm for healthy and mastitic
297 buffaloes respectively, values similar to how found in our study.

298 In this study, clinically healthy buffaloes were enrolled to avoid bias results due to diseases such as
299 mastitis. In this regard, Hussain et al. (2013) carried out a *post-mortem* study on healthy and mastitic NR
300 buffaloes and analyzed teat traits like TD, TL, and TCL in infected and healthy quarters. The analysis of
301 variance revealed that mastitis can affect the teat anatomy. Overall, the TCL measured by Hussain et al. (2013)
302 was about 3 times greater than the TCL of this study which was measured using echography images. In fact,
303 TCL averaged 47.7 and 52.1 mm in healthy and mastitic quarters, respectively.

304 In terms of LSM, the NR breed showed the lowest TCL. This difference suggests that the breed needs
305 specific adaptations of milking devices and that milkability can be dramatically different compared to other
306 breeds. Peculiar NR anatomical characteristic should be taken into account when developing any mastitis
307 control program or designing specific milking equipment for this breed.

308 Finally, only partial information on teat anatomy is available for the AZ breed (Alkhateeb et al. 2021).
309 For example, no information is published for TCL. The average TL in AZ buffaloes has been reported to be
310 48.2 and 78.8 mm in front and rear teats, whereas TD averaged 30.8 mm in front teats and 42.0 mm in the rear
311 ones (Alkhateeb et al. 2021). This paper reports for the first time TCL, TW and CD measurements of the AZ
312 buffaloes and represents a new and important information for the characterization of the breed.

313

314 ***Other Fixed Effects***

315 In dairy cows, Klein et al. (2004) observed an increase in TD and TW with increasing parity, likely due
316 to the cumulative effect of repeated milking events which include exposure to mechanical stress that, in turn,
317 may increase thickness of the teat wall (i.e. TD; Hebel et al. 1979).

318 In a recent study, data of 59 Murrah buffaloes were used to evaluate the effect of parity on the teat and
319 udder morphological traits (Bharti Praveen et al., 2015). Authors observed that the traits differ according to
320 the buffaloes parity and therefore age. In general, TD and TL increased with parity, confirming the results
321 observed in the present study (Table 3).

322 Regarding the frontal teat position, i.e. position I, in our study significant differences were found only for
323 TD, CD and TL. Rear teats had greater LSM compared to front ones (Table 3) and, although several
324 explanations can be proposed for this trend, the most important reason is related to the teat capacity. Like in
325 dairy cows, rear teats, in fact, are slightly larger than the front ones and contain more milk. The approximate
326 ratio in buffalo as well as in cows is 60:40; this explains why milking the hind quarters takes longer in general
327 (Thomas, 2008).

328 Lateral position of the teats did not affect the anatomical characteristics of the teats. Although there is
329 scarce literature available to discuss this result, in Indian dromedary Kumar et al. (2023) found that the TD of
330 left posterior teats was greater compared to the other teats and that the right anterior teat was the one with the
331 lowest TD. It is important to consider that the effect of teat position can also depends on the milking methods

332 used. In dairy cattle, some authors suggested that the differences found between left and right teats may be
333 affected by the milking position (right- or left-side milking), which can cause a greater pressure on the rear
334 teats of one side with a progressively consequent increase in the teat size.

335 In this study, two breeds were manually milked (NR, EG) and the procedure adopted did not include the
336 so called 'knuckling' or 'stripping' (Tamil Nadu Agricultural University, 2009), an intentional action
337 performed with the fingers in the past to reduce resistance of the teat sphincter. This manual stress can cause
338 stretching and damage to the teats, with different effects on the internal and external anatomical traits of the
339 left and right teats. Although both manually milked, NR and EG presented different averages and LSM in our
340 study for TCL and TL, suggesting that the milking type does not mask other effects and does not overlap with
341 the effect of breed. Authors of this study recommend future studies comparing teat anatomy in buffaloes of
342 different breeds subjected to both mechanical and manual milking.

343

344 ***General Considerations***

345 Several studies conducted on large and small ruminants demonstrate that teats morphology and milkability
346 traits are connected. In fact, most of the udder health selection indexes of dairy cows put emphasis on mammary
347 gland morphological characteristics. Apart from Italy, where genetic selection is currently active and
348 monitored by ANASB, in other buffalo populations, selection towards milk yield and/or mastitis resistance is
349 not officially pursued on a large scale level. Accurate phenotypes and pedigree data are fundamental for
350 making breeding decision and for establishing robust mating plans, but the collection is difficult to implement,
351 especially in some areas and contexts.

352 Correlated traits are useful indicators for breeders, as they can be used for genetic improvement.
353 Correlations between teat anatomical traits and milkability and between teat anatomical traits and
354 mastitis/udder health are reported in literature for different dairy species. In dairy cows, for example, Weiss et
355 al. (2004) observed that while the flow rate of milk was positively correlated with TD, the same was negatively
356 correlated with TCL. Bobic et al. (2014) recorded data on both Simmental and Holstein Friesian cows and
357 found significant differences between teat anatomy and milking traits in the two breeds and calculated
358 significant correlations between TCL and milk flow rate (negative) and between TCL and milking time
359 (positive), indicating that a higher milk flow is associated to a shorter milking session.

360 In dairy camel, Atigui et al. (2014) reported positive correlations among udder characteristics, teat
361 measurements and milkability traits. In particular, a significant positive association was observed between TD
362 and udder depth and between TD and several milkability traits. Findings of Atigui et al. (2014) indicate that
363 camels with larger teats are milked more easily, which could be due to larger teat canals or lower resistance of
364 the teat sphincters and higher intra-mammary pressure due to higher milk in the udder.

365 In buffaloes, several authors (Boselli et al. 2010, 2014; Costa et al. 2020; Thomas et al 2004; Napolitano
366 et al. 2022) studied the association between the main teat anatomical traits and milk flow. In buffalo, there is
367 a great resistance of the muscle walls of the teat (Ambord et al. 2009; Borghese et al. 2007; Thomas et al.
368 2004), meaning that higher vacuum pressure is required during mechanical milking to open the teat canal and
369 reach the plateau phase compared to cattle. This peculiar characteristic is important to consider when preparing
370 milking protocols and machineries. Ambord et al. (2009) reports that buffaloes adapt more difficultly to
371 mechanical milking compared to cows, likely due to the presence of longer teat canals. A possible approach
372 to investigate the influence of teat anatomy on milkability is to measure the vacuum required to open the teat
373 canal (VO) which is 20 kPa in dairy cows. In dairy cows, the correlation between VO and TCL was positive
374 and significant (0.82) as well as the one between VO and TW (0.62); on the other hand, negative was the
375 association between VO and CD (-0.65) (Weiss et al., 2004). Given these associations, it is reasonable to
376 assume that TCL and other teat anatomical traits, can have an influence on milk ejection consequently affecting
377 the milkability.

378 Teat anatomy, especially TCL, is crucial for the defense mechanisms against bacterial colonization of the
379 teat canal. Cobirka et al. (2020) showed how the teat end's level of defense against pathogens depends upon
380 several specific physical and physicochemical factors including, among others, TCL, amount of keratin
381 present, and milk flow rate. It is authors' belief that the long TCL of buffaloes (more than double compared to
382 dairy cows) has an indirect beneficial effect on udder health, mastitis resistance, and milk somatic cell count.

383
384 In conclusion, a better understanding of the main anatomical teat parameters of the most popular buffalo
385 breeds raised in the world (MI, AZ, BM, EG and NR) is useful to characterize the population, define reference
386 values, and therefore easily identify anomalies. Moreover, studying the effect of parity and teat position is also
387 pivotal for proper evaluation of anatomical characteristics and for decision-making. Mammary gland

388 morphology is heritable and associated with udder health in dairy breeds/types, therefore, a deeper knowledge
389 of phenotypic variability can support breeders to guide possible selection plans. Although this work represents
390 a preliminary investigation due to the small number of animals and herds involved, it is recommended to
391 validate findings in future improving representativeness of farm management and systems by including a larger
392 number of herds.

393 Authors of the present study expect that in the coming decade these findings would be useful to breeders:
394 the focus on functional traits like udder health is expected to increase in buffalo, in parallel with the demand
395 of milk and dairy foods, especially in African and Asian countries where the population is dramatically
396 increasing along with the food demand. Other than accounting for productivity level, the genetic selection and
397 improvement of buffaloes should aim to boost milk quality, fitness and health, relying on easy-to-measure
398 proxies - like teat measurements and milk composition. Findings advise that any breeding objective or indicator
399 trait/proxy for selection programs to improve udder health of buffaloes should be tailored according to the
400 breed-specific mammary gland morphology, productivity level, and susceptibility to clinical or subclinical
401 mastitis.

402

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412 **Author contributions**

413 Conceived of or designed study: the experimental design and data collection protocol were conceived, planned
414 and developed by Antonio Borghese, Angela Costa and Carlo Boselli.

415 Performed research: all authors contributed to the measurement and data collection in the field.
416 Analyzed data: Antonio Borghese, Angela Costa and Carlo Boselli performed the statistical analysis and
417 interpretation of the results.
418 Wrote the paper: the primary research draft was written by Antonio Borghese, Angela Costa and Carlo Boselli.
419 Angela Costa and Carlo Boselli improved and corrected the manuscript. All authors discussed the results and
420 commented on the manuscript. The final version of the manuscript was read, improved and approved by all
421 authors.

422

423 **Data availability**

424 Data will be made available on reasonable request.

425 **Declarations**

426 **Ethics approval**

427 The ultrasound teat measurements were performed by veterinarians, in accordance with internationally
428 accepted standard ethical guidelines for the use and care of animals.

429 **h) Conflict of Interest Statement**

430 No conflicts of interest.

431 **Competing Interests**

432 The authors have no relevant financial or non-financial interests to disclose.

433

434 **g) References**

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