

Ultrasonographic Evaluation of Teat Structure and Detection of Prominent Annular Folds in Brazilian Dairy Buffaloes

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Abstract: Udder and teat characteristics differ between buffaloes (*Bubalus bubalis*) and cattle (*Bos taurus*) and the differences are important in relation machine milk ability. Dairy buffalo milk out more slowly and are more difficult to milk than dairy cows, especially when milking machines are used. The goal of this study was to determine the ultrasonographic characteristics of udder and teat morphology in dairy buffaloes in Brazil, a country with a relatively recent history of buffalo farming. External assessment of the udder type and ultrasonography of the teat were done in 63 purebred Murrah and Mediterranean × Murrah dairy buffalo cows on three farms with different management systems. Udder types differed among farms; the rear-heavy udder type was most prevalent on two farms and the ball-shaped type occurred frequently on the third farm. Ultrasonographic measurements of the teat were made on one farm and showed correlations between the width of the teat tip and teat canal length, between the width of the teat tip and teat wall thickness and between the width of the teat tip and teat length. During the ultrasonographic examination of the teat, hyperechogenic membrane-like structures in the region of the annular folds, referred to as prominent annular folds (pAFs), were detected in at least one teat in 98 % of all buffaloes. These folds caused narrowing of the lumen between the teat cistern and gland cistern, but their physiological function and relevance concerning machine milk ability remain unclear. Further studies are needed to evaluate the effect of prominent annular folds on milk flow characteristics during mechanical milking in dairy buffaloes.

Keywords: Dairy buffalo, udder, teat structure, prominent annular folds, ultrasound.

INTRODUCTION

Buffaloes contribute significantly to milk production in many countries. Buffalo farming is relatively new to agriculture in Brazil; it is believed that the first buffaloes were from Italy and arrived on the Island of Marajó in northeastern Brazil at the end of the 19th century. Since then, the population of buffaloes has increased to more than 2 million head [1].

Traditional buffalo milk production systems have varied widely, but there is now an increasing demand for mechanized harvesting of milk. Machine milking is common in countries with intensive buffalo farming such as India and Italy [2]. Compared with dairy cows, buffaloes are slow and difficult to milk, especially with milking machines [3]. Thorough knowledge of the udder and teat structure is crucial for successful mechanical

or automated milking methods. Anatomical and physiological characteristics related to milking in buffaloes include a smaller gland cistern [4], slow milk ejection [2] and teat closure due to collapsed tissue at the distal part of the teat cistern [5]. These features have been associated with problems during mechanical milking. However, studies on teat and udder structure in dairy buffaloes are scant and the knowledge relating to the difference in milking ease between buffaloes and dairy cows remains incomplete.

Ultrasonography is a useful non-invasive method to assess teat structure and has been used widely in dairy cattle [6]. Ultrasonographic characteristics of the teats of dairy cows and buffaloes correlate with milking characteristics [7,8]. Furthermore, udder morphology of dairy sheep, goats and cows correlate with milk yield and machine milk ability [9-11].

The primary goal of this study was to determine the characteristics of udder type and ultrasonographic teat structure in dairy buffaloes from a variety of farming

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systems in Brazil. The aim was to examine the relationship between udder type and certain ultrasonographic features of the teat.

MATERIALS AND METHODS

Buffaloes and Farms

Three dairy buffalo farms with different management systems and from different parts of Brazil were selected. Farm 1 was an organic farm in southern Brazil (Paraná) with 25 lactating buffalo cows. Farm 2 was a conventional farm in a rural area in the Amazonas region (Pará) with 42 lactating buffaloes, and farm 3 was a newly established operation with 180 buffalo cows in north-eastern Brazil (Pará). All buffaloes were kept on pasture year-round and calving was mainly seasonal (March-June). The concentrate was fed during milking according to individual production levels. Farm 1 used a simple home-made milking parlour and a portable milking machine. In farm 2, cows were milked by hand in four home-made milking parlours. Farm 3 had a herringbone milking parlour with a manual detaching system. All cows were milked once daily in the morning. In farm 1, some of the calves accompanied the cows during milking and in farm 2, all the calves were present during milking. Calves were kept separate from their mothers overnight. In farm 3, calves were not present during the milking process. Milk yield of individual buffalo cows was measured once per week on farm 1 and 3 but not on farm 2. Performance data (milk yield, age and DIM) of the buffaloes from farms 1 and 3 are shown in Table 1.

Study Design

A total of 63 buffalo cows that were clinically healthy (Dirksen *et al.*, 2012) and had no external teat injuries or clinical mastitis, were selected and examined by ultrasonography immediately before milking. The study was performed under the consideration of the guidelines for the treatment of animals in behavioural research and teaching [14].

In farm 1, all lactating cows were examined and on farms 2 and 3, the cows that entered the milking parlour first were used. This yielded 20 cows on farm 1, 21 cows on farm 2 and 22 cows on farm 3. The cows were not selected for days in milk (DIM), milk yield or age. In farm 1, the cows were examined immediately before milking in a parlour used for treatment, which was separate from the milking parlour. On farms 2 and 3, examinations were carried out in the milking parlour, as there was no treatment parlour available. All buffaloes were evaluated once by an experienced examiner (LB). In farm 1, udder type was assessed visually, the teat and gland cisterns were examined ultrasonographically and ultrasonographic measurements of the tip of the teat including the teat canal were made. On farms 2 and 3, the udder and teats were assessed in the same fashion but no measurements at the teat tip were made.

Assessment of Udder Type and Measurement of Teat Length

The udder type was assessed according to Coban *et al.* (2009). Three udder types were distinguished: a *pendular udder* was bag-shaped and had a narrow attachment to the body of the buffalo; a *rear-heavy udder* had fore udder with a base that was situated higher than that of the rear udder; and a *ball-shaped udder* resembled a hemisphere when viewed from the side, and had a relatively narrow attachment to the body of the cow (adapted from Coban *et al.*, 2009). The length of the teats was assessed in 20 cows on farm 1 using a measuring tape.

Ultrasonographic Evaluation of the Teat Tip and the Teat and Gland Cisterns

Three different ultrasound machines (Echo Camera SSD 500, Mindray Z5Vet, Mindray DP20) equipped with a 4.5 or 5 MHz rectal linear transducers were used depending on their availability at the respective veterinary clinics. For evaluation of the teat canal and measurements of the distal part of the teat, the teat was immersed in a plastic cup filled with warm water

Table 1: Performance Data of 63 Lactating Buffalo Cows

	Farm 1	Farm 2	Farm 3
Number of cows examined	20	21	22
Mean daily milk yield in kg	7.5 (min, 4.2; max, 12.8)	No data available	2.9 (min, 1.0; max, 6.0)
Mean age of cows (years)	5.6 (min, 3.0; max, 12.0)	No data available	6.6 (min, 3.2; max, 13.5)
Mean days in milk	64.9 (min, 26; max, 85)	No data available	187.5 (min, 61; max, 733)

and the probe was applied to the ultrasound gel-covered wall of the cup to prevent compression of the teat. For the cows of farm 1, the images were frozen on the screen, and *teat canal length*, *the width of the tip of the teat* (at the level of the rosette of Fuerstenberg) and *teat wall thickness* approximately 1 cm proximal to the inner teat canal orifice were measured. During measurements at the teat tip on farm 1, structures at the border between the teat cistern and the gland cistern in the region of the annular folds were detected. These structures are hitherto referred to as *prominent annular folds* (pAFs). All hyperechogenic structures at the basis of the teat that protruded from the teat wall into the cisternal space were considered pAFs. The area where these structures occurred was further examined in cows on farm 2 and 3 by applying the probe directly to the udder after application of ultrasound gel.

Statistics

Statistical analyses were done using SAS (version 9.2; SAS Institute Inc., Cary, NC). Milk yield, DIM and teat measurements were considered quantitative data, and udder type, the occurrence of pAF (in 0, 1, 2, 3 or 4 quarters) and age (≤ 6 years, > 6 years) were considered categorical data. Descriptive statistics were calculated using proc means. In farm 1, correlations among teat measurements were analysed using Pearson's coefficients of correlation (proc corr). Teat measurements of fore and rear teats were compared using a linear mixed model with a random animal effect

(proc mixed) to account for repeated measurements within cows. The occurrence of pAFs was described at the animal level and the teat level. Fisher's exact test was used to examine the significance of the association between the categorical variables udder type and fore or rear udder and the occurrence of pAFs. The effects of the occurrence of pAFs, udder type, age and farm on milk yield were analysed using a linear model and F-test on fixed effects (proc mixed). The variable DIM was not included in the model because there was no apparent relationship between the stage of lactation and milk yield. The effect of the farm, age and udder type on the occurrence of pAFs in 0, 1, 2, 3 or 4 quarters was investigated using the F-test and a generalised linear model with pAF as the response variable with the binomial distribution. Residuals of the quantitative variables in the analysis of correlation and linear models were tested for normality using the Shapiro-Wilk test (proc univariate).

RESULTS

Udder Type and Ultrasonographic Teat Measurements

The distribution of udder types on the three farms is shown in Table 2. Farm 1 had a large proportion of *rear-heavy udder* types but the other types were also seen. *Rear-heavy udders* were also predominant on farm 2, whereas *ball-shaped udders* had a high prevalence on farm 3. The results of the teat measurements made in farm 1 are shown in Table 3.

Table 2: Distribution of Udder Types in 3 Brazilian Dairy Buffaloes Farms

	Farm 1	Farm 2	Farm 3
Number of cows	20	21	22
Udder type:			
Ball-shaped	6	3	13
Rear-heavy	12	18	3
Pendular	2	0	6

Table 3: Ultrasonographic Teat Measurements (cm) in 20 Lactating Buffalo Cows (80 Teats) on Farm 1 and Comparison between Rear and Fore Teats (LSMean \pm SE)

Variable	Rear teats	Fore teats	P
Teat length	9.9 \pm 0.30	8.7 \pm 0.31	0.01
Teat canal length	1.5 \pm 0.10	1.4 \pm 0.10	0.20
Width of teat tip	2.4 \pm 0.07	2.2 \pm 0.07	0.01
Teat wall thickness	0.72 \pm 0.04	0.71 \pm 0.04	0.80

There were significant correlations between teat canal length and width of the teat tip ($r = 0.60$; confidence interval (CI) 95% = 0.44; 0.72), between the width of the teat tip and teat wall thickness ($r = 0.357$; CI 95% = 0.15; 0.54) and between the teat wall thickness and teat length ($r = -0.28$; CI 95% = -0.47; -0.06).

The Occurrence of Prominent Annular Folds

Ultrasonographic evaluation of the teats of cows on farm 1 led to the coincidental detection of pAFs (Figure 1), which were also detected on farm 2 and 3 (Table 4). There were significant associations between the occurrence of pAFs and position of the teat (fore or rear udder) on farm 1 ($p = 0.01$) and farm 3 ($p = 0.03$) but not on farm 2 ($p = 0.66$). On farms 1 (30 rear and 17 fore teats affected) and 3 (35 rear and 23 fore teats affected), pAFs were more common in the hindquarters

than in the forequarters. The occurrence of pAFs was not associated with milk yield ($p = 0.82$), age of the buffalo ($p = 0.99$) or udder type (farm 1, $p = 0.79$; farm 2, $p = 0.36$; farm 3, $p = 0.48$). Milk yield was associated with farm of origin ($p = 0.03$); cows on farm 1 produced significantly more milk ($P = 0.003$) than cows on farm 3 [Least Square Means (SE) farm 1: 7.63 (0.66), farm 2: 3.22 (0.86)], but was not affected by age of the cow ($p = 0.40$) or udder type ($p = 0.38$). The presence of pAFs on 0,1,2,3 or all 4 quarters tended to be different between farm 1 and 3 ($p = 0.10$). The factor farm (analysed only for farms 1 and 3) had a small effect on the occurrence of pAFs; there was a trend for more cows with 3 or 4 pAFs on farm 3 (probability 0.76) than in farm 1 (probability 0.49; $p = 0.10$). These observations were only made for farm 1 and 3 as there were no management data available for farm 2.

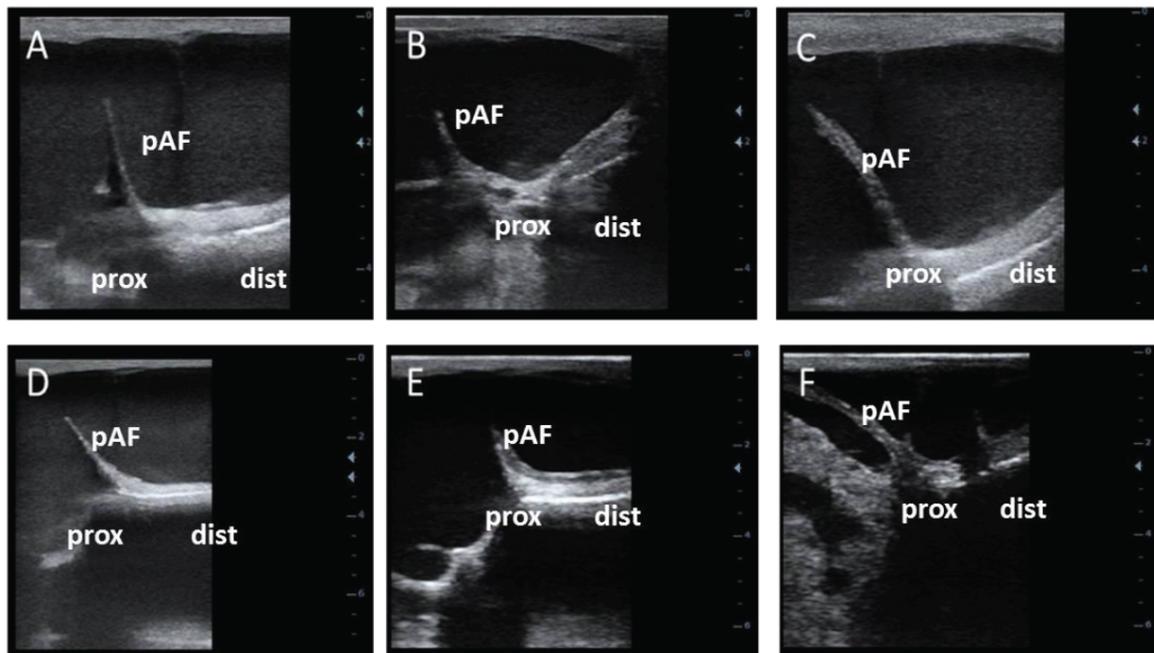


Figure 1: Ultrasonograms of teats of Brazilian dairy buffaloes showing prominent annular folds at the transition between teat cistern and gland cistern (prox = proximal, dist = distal). Ultrasound machines with 4.5 or 5 MHz rectal linear probes were used to scan the teats.

Table 4: Occurrence of Prominent Annular Folds (pAF) and Number of Teats Affected in Lactating Buffalo Cows

	Farm 1	Farm 2	Farm 3
Number of cows	19	19	17
No pAF	1	0	0
pAF in 4 teats	5	1	6
pPAF in 3 teats	1	2	6
pAF in 2 teats	9	10	2
pAF in 1 teat	3	6	3

DISCUSSION

The present study describes udder types and teat structures in Brazilian buffalo cows kept under various management conditions. The classification of udder types seen in dairy buffaloes is inconsistent in the literature and many different types have been described, including the globular, pendular, goaty, bowl [16,17] and trough type [18]. However, the exact defining criteria for each type are not consistently provided. For the present study, udder types were divided into pendular, rear-heavy and ball-shaped types, which had different distributions on three farms. Udder type is assumed to be a genetic trait, and the preference and management needs of the farmer as well as the availability of milking buffaloes ultimately determine which udder type predominates in a herd. Udder type traits have been associated with milking traits and udder health [19,20]. In first lactation Holstein cows, udder depth, udder balance (low or high rear quarters) and udder attachment were favourably associated with somatic cell counts and incidences of clinical mastitis [20]. In our study, we only used 3 different udder types, from which probably the ball-shaped udder type is the one with the most favourable udder traits as a tight and broad udder attachment and a good balance between fore and rear quarters. The rear-heavy type was predominant on farms 1 and 2. Farm 1 had a simple milking system but high-producing cows. The farm had been in operation for several years without much change in the genetic makeup of the herd, and udder type had not been considered an important selection criterion. Farm 2 had a very basic management system, which included milking by hand, and selection criteria are not reported. In contrast, farm 3 was a recently established, modern and well-managed operation with a herd that consisted of cows from all over South America. Selection of cows was heavily based on milk production and body type, and most of the cows had ball-type udders. The ball-type udder type was also the most prevalent udder type in a study on Asian Murrah buffaloes [17]. There are few studies on udder and teat measurements in buffaloes, but this information would aid in understanding how they correlate with milk yield and milk ability. Correlations between udder morphology and milk yield and machine milk ability have been reported for dairy sheep, goats and cattle [9–11]. Analogous data in buffaloes would facilitate the selection of milking buffaloes, especially because mechanical milking continues to pose difficulties in this dairy species. Studies in buffaloes found an association between

certain teat structures and machine milk ability. Teat canal length measured ultrasonographically was positively correlated to milk flow and milking time in Mediterranean buffaloes [8]. Ultrasonographic evaluation of the teat anatomy of Mediterranean buffaloes revealed a collapsed section of the teat cistern proximal to the teat canal, which was thought to function as an extension of the teat canal. The authors speculated that these structures were responsible for the increase in time required for pre-stimulation of the udder before milk let-down compared with dairy cows [5]. Those authors reported a teat canal length of 23.6 mm before stimulation and 14.8 mm after stimulation of the udder, which was longer than the lengths measured in the present study. Likewise, teat wall thickness measured in the present study was smaller than the results of a previous study [5]. It is possible that handling of the buffaloes as separation of the herd in a separate treatment parlour and ultrasonographic examination of the teat on farm 1 in the present study had a stimulatory effect on milk ejection, which may have reduced teat canal length as well as teat wall thickness [5]. Teat wall thickness was negatively correlated with teat length; long teats had thinner walls than short teats. No such correlation occurred in Mediterranean Italian buffalo [8] or dairy cows [7], and the relevance of our findings is not known to date.

During the ultrasonographic teat examinations on farm 1, pAFs were observed at the transition of the teat cistern to the gland cistern in several cows. We were unable to find a description of these folds in the literature and surmised that they may be attributable to a genetic defect caused by inbreeding in this particular herd. A pedigree analysis could not be performed as data were not consistently available on the investigated farms and the number of animals was too small. To clarify this phenomenon, ultrasonographic examinations of buffalo teats were conducted on two other farms in different parts of Brazil with different management systems and cows with different genetic makeup. To our surprise, pAFs were also seen in most of the cows from farms 2 and 3. Interestingly, pAFs were more common in the rear quarters than in the forequarters in cows from farms 1 and 3, but the reason for this is not known. Annular folds are considered to be folds of the mucosa [21] or to consist of elastic tissue [22] that divides the lactiferous sinus into a glandular part and a papillar part [23,24]. An older study suggested that milk let-down may be blocked by contraction of smooth musculature at the base of the teat, including the annular folds. A case

report of a perforating teat injury in a Shorthorn cow described that after the udder was stimulated, the flow of milk from the perforation could be completely stopped by touching the teat. The author concluded that there must be a reactive barrier between teat and gland cistern which allows "holding" of the milk [25]. It is conceivable that the annular folds are involved in the inhibition of milk let-down. The teat wall contains smooth musculature, which is regulated by the sympathoadrenal system [26]. Ultrasonography showed that when alpha-adrenergic receptors are stimulated by substances such as phenylephrine, the size of the cisternal area decreases [27]. Involvement of the annular folds in the regulation of the cisternal size has not been reported.

A study that described the ultrasonographic appearance of the udder and teats in buffaloes identified anechoic areas in the region of the annular folds consistent with blood vessels belonging to the papillary venous plexus. Other structures were not seen in that region [28]. A distinct anatomic feature of the gland cistern of buffaloes referred to as pouches were described in a study that compared the mammary tissue of buffaloes and dairy cattle. This feature was not the same as the annular folds but occurred in the same region of the cistern [29]. The relevance of pAFs observed in the present study is not clear. They may represent an additional protective mechanism in the mammary gland, which would be beneficial for buffaloes because they spend extended periods standing in dirty water, but this theory has not been tested. In dairy cattle, stenoses in the region of the annular folds have been shown to affect milk flow [30]. Buffaloes are slow and difficult to milk compared with dairy cattle [3,31] because the cisternal volume is smaller, milk let-down is slower and there is an additional teat closure mechanism [3,5,31,32].

CONCLUSION

The detection of pAFs in buffalo teats is novel and has never been described before. It is not clear from the findings of the present study whether pAFs affect machine milk ability and whether their formation is induced by external stimulation of the udder. Therefore, the function of pAFs and their effect on milk flow characteristics in buffaloes requires further investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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