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Effect of Season and Age on Bacterial Load in Fresh Semen Ejaculates of Buffalo Bulls

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ABSTRACT

Fresh semen of 10 Murrah buffalo bulls of two different age group viz. 4-5 years and 8-9 years having five bulls in each, were evaluated for total viable bacterial load during three different seasons (summer, rainy and winter).Mean bacterial load in fresh ejaculates of buffalo bulls (three ejaculates per bull in each season) was measured using standard plate count method and expressed as mean (± SEM) CFU per ml of semen. Non significant (P > 0.05) but visible differences of mean bacterial load in bubaline semen was recorded between seasons. Comparatively higher bacterial count in bubaline semen was observed during summer season (18000 ± 4667 CFU/ml) as compared to rainy (16000 ± 3055 CFU/ml) and winter season (12000±3266 CFU/ml). Likewise, nonsignificant (P>0.05) seasonal influence was recorded over bacterial load in semen from bulls of two different age groups. However, appreciable variation was noticed within seasons. During summer season, younger bulls (aged between 4-5 years) showed higher bacterial load (22000±8000 CFU/ml) as compared to older bulls (14000±5099 CFU/ml) and vice versa during winter season. Variations recorded during present study was due to significant variation (P<0.05) among individual bulls within and between seasons. Bacterial load in bubaline semen during summer season was negatively correlated with that during rainy (r = -0.296) and winter season (r = -0.19), however positive correlation (r = 0.423) was noticed between rainy and winter season. In conclusion, the changing seasons during the period of study produced nonsignificant effect on microbial quality of the spermatozoa in Murrah bulls.

Keywords: Season, Age, Murrah bulls, Semen, Bacterial load

The buffaloes are in the order of *Artiodactyla*, the cloven-hooved mammals, genus *Bubalus* and species *bubalis*. The buffalo population is continuously increasing, and is estimated at over 185.29 million head (FAO, 2008). 97 percent of them are water buffaloes and are mainly found in the Asian region. India has 105.1 millions and they comprise approximately 56.7% of total world buffalo population. Riverine buffaloes (70 percent of the total world population) of well defined breeds are found only in India and Pakistan (Drost, 2007). Murrah buffalo is the most efficient milk producer and has better adaptability throughout India (Resali, 2000). The production potential of local nondescript/ Murrah buffaloes in Chhattisgarh are being increased by genetic improvement through artificial insemination (AI) using cryopreserved semen of Murrah bulls. Seasonality in buffalo reproduction has been reported from India, Pakistan and other parts of world (Singh *et al.*, 2013). Although buffalo bulls breed the year round, conflicting reports have been published about semen quality at different ages and during various seasons of the year. Studies examining the relationship between climatic changes and some parameters of semen have been already published for Murrah (Bhosrekar *et al*., 1992), Surti (Gupta *et al*., 1978), Nili-Ravi (Javed *et al*., 2000) and swamp buffalo (Koonjaenak *et al*., 2007a), but none of reports discussed the influence of season on bacterial load in bubaline season. The bacterial contaminants of semen have been a major concern for most semen production laboratories as it adversely affects the semen quality and hence the subsequent fertility (Grieveu *et al*., 1995). It

is well known that presence of bacteria in the ejaculates can affect fertilization directly (Morrell, 2006), impairing their motility (Bussalleu *et al*., 1986) and increasing DNA fragmentation rates (Gonzalez-Marin *et al*., 2011). As the season of our country and Chhattisgarh, in particular varies markedly, special attention should be given to the fertility of bulls, all the year round. The objective of the present study was, therefore undertaken to determine the bacterial load in bubaline semen during three different seasons.

MATERIALS AND METHODS

Location

Present study was conducted at Central Semen Station, Anjora, District-Durg situated on (between 17-23.70 N Latitude and 80.43-83.380 E Longitudes) western part of Chhattisgarh state in India. Semen Station, in particular is located on the bank of river Shivnath.

Distribution of Season

Meteorological data of Durg district was taken from report of National Horticulture Commission, Department of Agriculture and Cooperation, Krishi Bhavan, New Delhi (NHC, 2013) and official website of Durg district (durg.gov.in/District profile_General.html). Durg generally has a dry tropical and sub-humid type climate which is moderate; however summer is a little bit hotter. Rise of temperature begins from the month of March to May. The peak temperature reaches in May/June and can be as high as 48°C. Durg district's annual average rainfall is 1052 mm. The onset of monsoon is usually from June and the season extends up to September, with monsoon peaking during July and August. District receives very short winter season starting form last of November to mid of January with minimum temperature of 10°C. The atmospheric humidity is very high (>90%) during monsoon months and starts decreasing form October onwards and reaches as low as 15-20 percent during peak summer months. So, climate of Durg district was categorized into three main seasons i.e. summer (March to Mid June), winter (November to January) and rainy season (Mid June to September).

Experimental Animals

The study was conducted on 10 Murrah buffalo bulls (*Bubalus bubalis*) available at Central Semen Station, Anjora. Available bulls were categorized into two age groups viz. bulls aged between 8-9 years (G1) and between 4-5 years (G2), with five bulls in each group.

Table 1. Effect of season on bacterial load in semen of Buffalo.

S No	Age groups	Bull no.	Bacterial load(±SEM) CFU (x 100) /ml of semen		
			Summer	Rainy	Winter
1		555	100 ± 57^{ab}	100^{ab}	200 ± 57^{ab}
2		429	0^{a}	100 ± 57^{ab}	100 ± 57^{ab}
3	G1	525	200 ± 57^{abc}	200 ± 100^{bc}	200 ± 115^{ab}
4		559	100 ± 57^{ab}	300 ± 57^{c}	300 ± 100^{b}
5		560	300 ± 100^{Abc}	100 ± 57^{ABab}	0^{Ba}
Average Total (G1)			140±50	160±40	160±50
1		43712	400 ± 57^{Ac}	200 ± 57^{ABbc}	100 ± 100^{Bab}
2		116627	100 ± 57^{ab}	200^{bc}	200 ± 57^{ab}
3	G2	114161	200 ± 57^{Aabc}	0^{Ba}	0^{Ba}
4		81027	0^{Aa}	300 ± 57^{Bc}	0^{Aa}
5		44816	400 ± 152^{c}	100 ± 57^{ab}	100^{ab}
Average Total (G2)			220±80	160±50	80±37
Mean total of all bulls			180±46	160±30	120±32

Values given for individual bull in each season is the mean value obtained from three ejaculates.
Values with different superscript (a, b,c) with in a column differ significantly at 1% level (P<0.01).
Values with different superscript (A, B) with in a row differ significantly at 5% level (P<0.05).

Feeding and Management of Bulls

Feeding and managemental system as recommended in minimum standard for production of bovine semen (NDDB, 2012) was practiced in semen collection center. All the bulls were kept under identical conditions of management, feeding (seasonal fodder) and watering. The bulls were housed individually in pens with sufficient cross ventilation and protection against summer heat and in an open space for sunbathing in winter. General health conditions of all bulls were good.

Samples

Fresh semen ejaculates from all the bulls were collected early in the morning before sunrise with the aid of

an artificial vagina using routine collection technique (Shukla, 2008). Overall nine ejaculates (three ejaculates in each season) were taken from each bull during the whole year; March 2011 to February 2012. Samples were being collected from a bull keeping an interval of at least 10 days between two samples. Fresh semen samples were processed immediately for bacteriological examination within one hour after collection.

Table 2. Pearson correlation between seasons of bacterial load in bubaline semen

Age group	Season	Summer	Rainy	Winter
All ages	Summer	1	-0.296	-0.19
	Rainy	-0.296	1	0.423
	Winter	-0.19	0.423	1
G1	Summer	1	-0.49	-0.423
	Rainy	-0.49	1	0.784
	Winter	-0.423	0.784	1
G2	Summer	1	-0.441	0.2
	Rainy	-0.441	1	0.157
	Winter	0.2	0.157	1

Determination of Bacterial Load

The bacterial load in the semen samples was measured using the standard plate count method (Shukla *et al*., 2011). Culture media and reagents of HiMedia were used throughout the study.

As a procedure, 0.1 ml of freshly collected semen was added into a test tube having 0.9 ml of nutrient broth step wise to obtain: 10, 1: 1000 and 1: 10000 dilutions. By using double set of Petri dishes for each dilution, 0.5 ml from each diluted samples was spread on standard plate count agar. The Petri dishes were then incubated at 37°C for a period of 72 h, and the number of colonies that arose were counted by colony counter. The number of bacteria present in each Petri dish was calculated by multiplying the number of colonies with the dilution rate at which the colonies developed.

Data Recording and Statistical Analysis

Data were expressed as means (±SEM) colony forming unit (CFU) /ml of semen and analyzed by applying GLM (General Linear Model) for factorial experiments using SPSS computer software package (Version 16.0.0.247 ©2007). DMRT was done to make specific treatment comparisons for values that were found significant by ANOVA according to procedure outlined by Steel and Torrie (1980). Pearson's correlation between seasons; and age was calculated using bivariate (r_{12}) analysis.

Table 3. Pearson correlation between ages with in season for bacterial load in bubaline semen

Season	Age group	G1	G2
Summer	G1	1	0.564
	G2	0.564	1
Rainy	G1	1	0.294
	G2	0.294	1
Winter	G1	1	-0.629
	G2	-0.629	1

RESULTS AND DISCUSSION

There was nonsignificant (P>0.05) differences of mean bacterial load in semen of Murrah buffalo between seasons (Table 1), which may be correlated with the observation of Javed *et al*., (2000) and Saeed *et al.*, (1990) who reported non-significant difference in progressive motility of bubaline sperm between seasons. Above facts were supported by findings of Shukla (2005) and Azawi and Ismaeel (2012) who observed highly significant negative (P<0.01) correlation of standard plate count with progressive sperm motility and sperm viability, respectively. Similarly, Koonjaenak *et al*., (2007b) did not find any significant variation in DNA fragmentation in bubaline semen due to seasonal change. Although variations were nonsignificant during present investigation, marked differences were noticed. Comparatively higher bacterial count in bubaline semen was observed during summer season (18000±4667 CFU/ml) as compared to rainy (16000±3055 CFU/ml) and winter season (12000±3266 CFU/ml). Variations observed during present study might be due to significant variation (P<0.05) of bacterial load in semen of individual bulls (bull no. 560, 43712, 11461 and 81027) between and within seasons. Seasonal effects are caused by several factors such as cleanliness, ambient temperature, relative

humidity, day length and food quality (Bawa *et al*., 2011). The ideal climatic conditions for growth and reproduction in buffaloes are: temperatures of 13–18°C combined with an average relative humidity of 55–65%, and a medium level of sunshine (Payne, 1990). High heat stress during summer is known to depress the thyroid activity which consequently results in weak libido of breeding bulls and poor semen quality (Kastelic, 2013) and is aggravated when accompanied by high ambient humidity (Marai and Haeeb, 2010). Semen gets contaminated with bacteria either internally through local/systemic infection or externally from environment in preputial orifice (Perumal *et al*., 2013). Bacterial population present in semen grow best at temperature of 20-40°C (Baron, 2004) and it was found that semen samples were being collected in the morning before sunrise with ambient temperature about 30-35°C in summer and 15-20°C in winter during course of present investigation, which could favour for growth of bacteria during summer and causes bacterial inhibition during winter season. Present finding support the earlier findings of, Azawi and Ismaeel (2012) who reported higher bacterial count in ram semen during summer. On contrary, Ghoneim *et al*., (2014) reported significantly higher bacterial load in camel during peak breeding season. In accordance with present observation higher progressive motility in winter was observed by Koonjaenak *et al*., (2007a) and inferior semen quality during summer by Bhosrekar (1980). During rainy season, it is more difficult to keep bull's bedding and alleys clean, with consequent increasing amount of dirt on legs, flanks and preputial orifice (Zucali *et al*., 2011), which could account for higher bacterial contamination in semen during rainy season as compared to winter season.

Although, no significant ($P>0.05$) variation between seasons of bacterial load in semen from two different age groups was recorded, but appreciable variation was noticed within seasons. During summer season, younger bulls (aged between 4-5 years) showed higher bacterial load (22000±8000 CFU/ml) as compared to older bulls (14000±5099 CFU/ml) and vice versa during winter season. Similarly, Saeed *et al.,* (1990) reported the best quality semen at 3-4 years of age during winter season. On contrary, Younis *et al*., (1998) reported higher individual motility in semen of buffalo bull during peak breeding season (winter). These differences in present study might be due to difference in tolerance ability of bulls of two age groups against environmental stress during summer. Higher bacterial count in older bulls during winter season may be related to senile changes in them (Younis, 1996), whereas contradictory findings during summer season might be the effect of individual bulls. Age effects during present study may also be compared with semen investigation performed in other species. Almquist *et al.,* (1949) and Brown *et al*., (1974) reported higher bacterial load in semen of younger and older cow bulls, respectively. Whereas, Ghoneim *et al*., (2014) reported higher bacterial load in middle aged camels (9-13 years) than young (4-8 years) and old aged (14-18 years) animals. Rainy season did not produce any variation in bacterial load among two categories of bulls during present study. However, the bulls of the present study were reared under more or less stable environmental conditions. Therefore, it is likely that the variation in the semen quality in the present study could be attributed to significant ($P<0.05$) differences observed between individual bulls.

Bacterial load in bull semen were correlated between different seasons (Table 2). In general, bacterial load in bubaline semen during summer season was negatively correlated with that during rainy ($r = -0.296$) and winter season ($r = -0.19$), however positive correlation ($r = 0.423$) was noticed between rainy and winter season. Above findings indicated that winter and rainy season could be considered as favourable period of semen collection for improving the success of AI programme in buffalo, whereas high heat stress during summer could account for the increased bacterial load and reduced viability of sperm. Similar pattern was reported by Bhavsar *et al*., (1989) and they concluded that fertility of semen collected during season from July to January (rainy and winter) was higher than ejaculates collected during February to June (summer). Similarly, summer seasons were negatively correlated with rainy ($r= -0.49, -0.441$) in both age group bulls, however correlation between summer and winter season was negative ($r= -0.423$) in older bulls and positive ($r = 0.2$) in younger bulls. Differences between age groups may be due to cumulative effect of individual bulls. Correlation of bacterial load in bull semen between ages within season was also recorded (Table 3). Total bacterial count in semen of old aged buffalo bull showed positive correlation with that in younger bulls during summer ($r = 0.564$) and rainy season ($r = 0.294$), whereas negative correlation during winter season ($r = -0.629$). So, it seems

that summer seasons produced more or less uniform effect over both group of bulls, whereas adaptability of bulls of two age groups varied during winter seasons.

It can be concluded from present study that although age of bull and seasons of year produced noticeable variations of bacterial load in bubaline semen but these differences were nonsignificant (P>0.05), indicating that bulls tolerated the changes in environmental temperature and relative humidity well in Chhattisgarh region of India. However, future research is required to explore the possible reasons behind observed seasonal variation of bacterial load in bubaline semen.

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