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ABSTRACT

Repeat breeding is one of the important problems in dairy sector in Bangladesh. This study was conducted to estimate the proportionate prevalence of repeat breeder cows and its frequency distribution in 120 cows from 15 selected household dairy farms in Hathazari Upazilla, Chittagong during the internship rotation of January to March 2018. A repeat breeding cow may be defined as cows failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities. Data at different levels was collected through questionnaire interview. All farms had history of the current repeat breeding of one or more animals. Overall, the animal level proportionate prevalence of repeat breeder was 25 % (N=120). Cows aged with >6-8 years (33%) and having uterine infection (52%) had significantly higher prevalence of repeat breeding than that of the counter group whereas cows with parity 3 (22.39%) were more prone to breed breeder. Uterine fluid samples were collected from those 30 repeat breeder cows to know the distribution of organisms. *Staphylococcus* (76%) and *E. coli* (13%) were commonly found on bacteriological culture. It is concluded that the overall animal level prevalence of repeat breeder was high. Older animal and uterine infection were likely risk factors for repeat breeder. *Staphylococcus* was found common organism in uterine fluid.

Keywords: Repeat Breeder, Risk factors, Hathazari

CHAPTER I: INTRODUCTION

It has been reported that reproductive disorders are responsible for remarkable economic losses to the dairy farmers in Bangladesh (Talukder *et al.*, 2005). Among the major reproductive problems that have direct impact on reproductive performance of dairy cows are abortion, dystocia, retained fetal membrane (RFM), pyometra, metritis, prolapse (uterine and vaginal), anoestrus and repeat breeding. They are classified as before gestation (anoestrous and repeat breeding), during gestation (abortion, vagina prolapse and dystocia) and after gestation (retained fetal membrane and uterine prolapse (Lobago F *et al.*, 2006). A repeat breeder is generally defined as any cow that has not conceived after three or more services, has normal estrus cycle, is free from palpable abnormalities, shows no abnormal vaginal discharges, has calved at least once before and is less than ten years old. Repeat breeding has also been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities, is a costly problem for the dairy producer Gustafsson H, Emanuelson U (2002). Repeat breeding is one of the major infertility problems in the herd. The incidence of repeat breeding in dairy cows, worldwide, ranges from 3 to 10%. Overall prevalence of repeat breeder cow in commercial dairying of Chittagong was 11.3% (Nath *et al.*, 2014). As repeat breeding occurs there are clearly increase the calving interval and as the calving interval increase the milk production also reduces. The twelve-month calving interval is advantageous for maximal milk yield per cow per year with good economic return (Opsomer *et al.*, 1996). There are many reasons for the repeat breeder syndrome but researchers can't identify a specific cause. Both infectious and non-infectious causes are responsible for that. Generally, non-specific infection of the genitalia is considered to be the main cause of repeated conception failure (Singh *et al.*, 1996) where there is an increase in the number of microorganisms and/or in their virulence. Bacterial infection is the most important among the various causes of the subfertility (Dholakia *et al.*, 1987). Such a condition may cause cervicitis or endometritis of various degrees, which in turn may lead to embryonic death and repeat breeding problems (Elliott *et al.*, 1968). Poor farm management, chromosomal aberrations, hormonal imbalance, anatomical defects of reproductive tract, improper timing of

insemination, inadequate estrus detection, improper semen handling, infertile bulls, poor nutrition and heat stress etc. all are non-infectious causes ((El-Khadrawy HH *et al.*,2011). When artificial insemination is used, some of the animals might have been inseminated at wrong time (Shamsuddin *et al.*, 2001) leading to increased proportion of repeat breeding in Bangladesh. Moreover, Jainudeen and Hafez (2000) reported higher incidence of repeat breeding in dairy herds using artificial insemination than that used natural services. Condition may cause cervicitis or endometritis of various degrees, which in turn may lead to embryonic death and repeat breeding problems (Elliott *et al.*, 1968) In infectious cause microbes sometime which are normal commensals may produce a marked change in pH of uterine and vaginal secretions, inflammation uterine mucosa and thereby interfere with the implantation of fertilized egg (Singh NP, Chaturvedi VK, Singh DP, 1996). The importance of microorganisms has been recognized as one of the causes of infertility ((Singh NP, Chaturvedi VK, Singh DP, 1996). Sometimes farmer used to inseminate two times for each heat, antibiotic used to increase fertility and hormonal treatment as well. Considering these, the study was designed to assess the presence of bacteria in the uterine environment of cows with the aims to isolate bacteria those are related with repeat breeding.

Chapter II

MATERIALS AND METHOD

The study was conducted in household dairy farms at Chittagong of Bangladesh during January to March 2018.

Selection of cows

A total of 120 cows from 15 household dairy farms were selected in this study. Farms were selected on the basis of simple randomization and owner's cooperation. Among the 120 cows, 30 were primarily identified as repeat breeder. Cows were considered as repeat breeder on the basis of following criteria including was not conceived after three or more services, was normal estrus cycle, was free from palpable abnormalities, shown no abnormal vaginal discharges, was calved at least once before and was less than ten years old. These criteria were detected by visual physical examination and rectal palpation of the cows. These primarily selected repeat breeder cows were also considered for cow level data collection and uterine sampling.

Questionnaire design and data collection

A structured questionnaire was constructed to acquire data regarding farm and cow level management, demographic, health, production and reproduction. The questionnaire was designed to comprise mostly closed and open ended face to face questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield MV, 2005). Important farm and cow level intended data related to repeat breeder syndrome were collected and recorded.

Collection and Culture of Bacteriological Samples

Uterine samples were drawn from repeat breeder cows with the help of a sterilized intrauterine catheter attaching 10 ml sterile syringe using negative pressure during the estrous period. Before going to farm for cow's uterine sample collection, owner's call was received over cell phone to know the cow was in estrus. Before taking

uterine samples from cows, the vaginal and perianal region are thoroughly washed with normal water and iodine solutions. After collection of samples were promptly transferred into sterilized 10 ml test tubes. The samples were then placed in an ice box containing ice cubes as early as possible and brought to laboratory within one hour. Each sample of uterine mucus inoculated in Nutrient agar (NA) and Blood agar (BA) to promote growth of bacteria. Each group of these media was incubated aerobically at 37°C. The colonies on primary cultures were repeatedly sub-cultured by streak-plate method (Cheesbrough, 1985) until the pure culture with homogenous colonies were obtained. Media such as NA, BA, Eosin methylene blue, Mannitol salt agar were used for these repetitive sub-cultures. The aerobic culture plates were incubated at 37°C in bacteriological incubator for 72 hours. The cultural examination of uterine discharge for bacteriological analysis was done according to the standard methods (Cowan, 1985) The examination followed detailed study of colony characteristics, cellular morphology and biochemical properties. The isolated organisms with supporting growth characteristics on various media were subjected to Gram's staining, motility test, and different biochemical tests described by a Farin (1989)

Statistical analysis

The data generated were stored in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, USA). Univariable regression models were used to compute the potential risk factors. Potential risk factors included in the univariable models were selected based on the existing literature. The potential risk factors (both cow and farm level) were screened through the univariate measures of association with P value of <0.05.

Chapter III

RESULTS

The frequency of RB in household dairy farm is represented in Table 1. A total of 120 cows were surveyed in 15 household dairy farms. The frequency of RB was 30(25%) and 90(75%) were normal cow.

Total No. of cow	Category	Frequency	Percentage (%)
N=120	Normal	90	75
	Repeat breeder (RB)	30	25

Age, lactation number, previous peripartum events were also studied and the results revealed that age >6-8 years and previous periparturient events like uterine infection had significant effect on RB syndrome. The cows belonging >5 to 6 yrs old and parturition without any abnormalities mean normal delivery showed lowest effects on RB syndrome. On the other hand, cows with more than 6 yrs old (33.92%) and those had uterine infection (52.38) resulted highest incidence of RB syndrome. Lactation number had no any significant effects on RB syndrome ($p \geq 0.05$). (Table 2)

Table 2. Association between RB and cow level exposures

Factors	Category	Repeat breeder (%)	Normal (%)	p
Age	Up to 4 year	7 (21.87)	25 (78.13)	0.05
	>5-6 years	4 (12.5)	28 (87.5)	
	>6-8 years	19 (33.92)	37 (66.07)	
Lactation number	LN=1	8 (29.63)	19 (70.37)	0.35
	LN=2	7 (19.45)	29 (80.55)	
	LN=3	15 (22.39)	42 (73.68)	
Peripartum events (last calving)	No event	3 (4.77)	60 (95.23)	0.01
	Dystocia	12(50.0)	12 (50.0)	
	Uterine infection	11 (52.38)	10 (47.61)	
	Abortion	4 (33.33)	8 (66.67)	

Besides the cow factors analyzed, farm level exposures were studied in this clinical study. Academic qualification of owner (0.05), drainage system at farm (0.03), visual hygienic score of cows (0.02) were identified as risk factors for incidence of RB. Nature of feed offer at farm (0.07), frequency and time of feeding (0.17), amount of green grass offered/day (0.27), person involved to insemination (0.17), RFM management practice at farm(0.39) have no significant effect on RB (Table 3)

Table3. Univariate association of RB with farm level exposures

Factors	Category	Repeat breeder (%)	Normal (%)	p
Academic qualification of owner	Up to Secondary	10(41.66)	14 (58.33)	0.05
	Higher Secondary	7 (31.82)	15 (68.18)	
	Graduate	13 (17.58)	61(82.42)	
Drainage system at farm	Good	7 (11.86)	52(88.14)	0.03
	Moderate	15 (34.88)	28(65.12)	
	Poor	8 (44.44)	10(55.56)	
Visual hygienic score of cows	Clean	14 (16.67)	70 (83.33)	0.02
	Dirty	16 (44.44)	20 (55.56)	
Nature of feed offer at farm	Mostly concentrate with irregular roughage	24 (24.24)	75 (75.76)	0.07
	Sufficient green grass with standard concentrate	6 (28.57)	15 (71.43)	
Frequency and	Twice(Morning-Afternoon)	11(21.15)	41 (78.85)	0.17

time of feeding	Twice (Morning-Evening)	19 (27.95)	49 (72.05)	
Amount of green grass offered/day	Up to 15	14(35.0)	26 (65.0)	0.27
	>15 kg/day	16(20.0)	64 (80.0)	
Person involved in Insemination	FAI(Govt.)	24(25.81)	69 (74.19)	0.17
	Private Technician	6 (22.22)	21 (77.78)	
RFM management practice at farm	Hormonal/Antibiotics	14(35.90)	25 (64.10)	0.39
	Manual Removal	16(19.75)	65(80.25)	

Primarily diagnosed repeat breeding cows were considered for uterine sample collection to isolation of organisms. Out of 30 uterine samples 2 bacterial isolates including *Staphylococcus* spp. 23(76%) and *E. coli* 4 (13%) were found (Fig. 1).

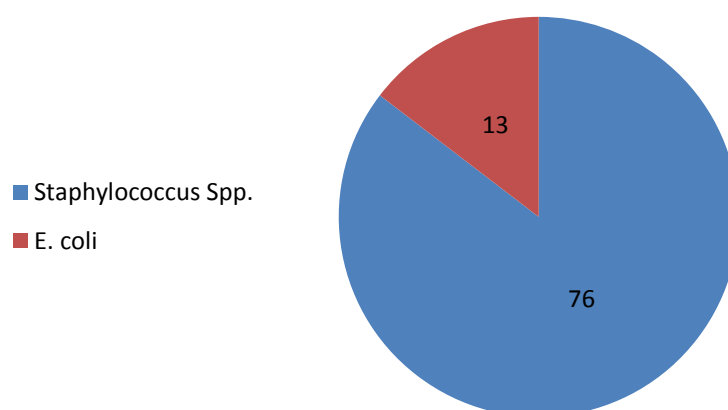


Fig1. Percentage of isolated bacteria in RB cow

There were two figures in the below .Figure3 was microscopic presentation of *E. coli* and Figure4 was microscopic presentation of *Staphylococcus* spp.



Fig.2 -Sample Collection

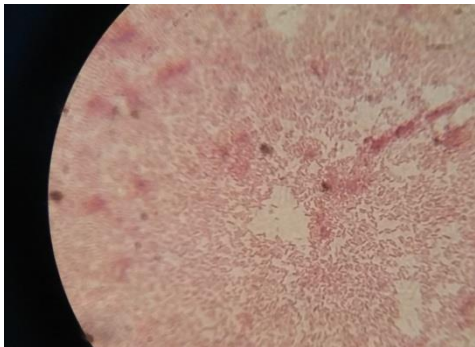


Fig3. -*E. coli* in Gram's Staining

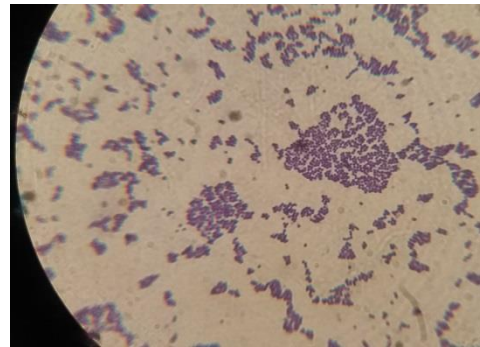


Fig4. - *Staphylococcus spp* in Gram's staining

Chapter IV

DISCUSSION

The present study recorded the overall prevalence of repeat breeding cows were 25%, which is in close agreement with the earlier findings of Motheshamuddin *et al.*, (2012). They showed 23% prevalence of RB in dairy cows. Again Sarder *et al.*, (2010) reported 20.2% incidence of RB which was slightly lower than the current findings. The variations might be due to the measures used to define RB, study design, difference in geographical location, agro-climatic zones and individual variations. According to previous report in our country, 13.0 to 22.0% cows was identified as RB (Samad, 1996).

In this clinical study, the factors related to cows at household dairy farms were found to significantly influenced as risk factors to incidence of RB. The cow age, lactation number and periparturient events at pervious parturition were studied. Age of the animals and problems in and around parturition significantly influenced the prevalence of RB ($p < 0.05$).

The prevalence of RB was highest 33.92% in >6-8 years cows which is a significant risk factor for RB whereas >5-6 years aged cows was affected with RB syndrome with least percentage (12.5%). According to findings of Gani *et al.*, (2008) RB rates were more prevalent in cows 7 years of age which is almost consistent with present study

In this study peripartum events of cows has significant effect ($p < 0.05$) on repeat RB syndrome. The study revealed that the prevalence of RB was higher among cows which had history of abortion (33.33%), dystocia (50%) and uterine infection (52.38%) during last calving in relation to no such type of event in previous parturition. Results of this study matches with the findings of another studies (Ariane *et al.*, 2011; Gustafsson and Emanuelson, 2002).

Lactation number was another factor studied which was not found as a risk factor on RB syndrome ($p \geq 0.05$). As there was a positive relationship between age of the cows and lactation number, therefore it was expected that lactation number have had influenced RB syndrome similar to age. The dissimilar results would be due to minimum number of data studied.

In this study farms owner's academic qualification was indicated as a risk factor ($p \leq 0.05$). Farm owned by a graduate having less (17.58%) affected with repeat breeder syndrome but farm owned by a educated up to secondary was affected highly (41.66%). There may be a reason of less affected with RB syndrome in graduate owned farm may be all about the hygiene and biosecurity as well as feed supplement and vitamin mineral supplement. The findings was not similar to the finding of the study by Nath *et al.*, 2014.

Drainage system of the farm was also identified as an important risk factor ($p \leq 0.05$). Farms having poor drainage facility was highly (44.44%) affected with RB but lower rate (11.86) in case of good drainage facility. Findings were found similar to that of Nath *et. al*, 2014.

Another risk factor identified in this study was visual hygiene ($p \leq 0.05$). If the cow clean then RB syndrome was only 16.67% in comparison with the dirty cows 44.44%. This visual hygiene indicates regular washing, removal of the manures after defecation so that manures were not in contact with the body surface. Findings were found similar to that of Nath *et. Al.*, 2014.

Type of feed offer at farm had not found any significant effect ($p \geq 0.05$) on repeat breeding. Green grass helps in folliculogenesis of ovary. β -carotene improved the pregnancy rate in RB cow by 33.3% compared with 27.2% in the control group which may be attributed to high oestradiol and progesterone levels resulting from increased follicle size and corpus luteum functionality (Celik *et al.*, 2009). Although green grass had effects but in this study the sample size was smaller and study was conducted on household dairy cows rather in commercial dairy farms. In household farms the owners sometime keep the cows in free range for a while so that may be a cause of disagree here

Some other factors such as frequency and time of feeding (0.17), amount of green grass offered/day(0.27), person involved in Insemination(0.17), RFM management practice (0.39) at farm were not identified as risk factors ($p \geq 0.05$) on RB syndrome.

Isolation of *Staphylococcus* spp., *E. Coli* spp. Were found from these repeat breeding cows. Huber (1982) found a positive correlation ($r=0.94$) between RB and bacterial infection of uterus. Some pathogens are commonly found in both normal and repeat breeder cows in various proportions, which indicate that these pathogens are

opportunistic to uterus. At present study, we found *Staphylococcus* spp. (77%), *Escherichia coli* (13%) in RB cows which in agreement with another study (Ahmadi *et al.*, 2007) who found *E. Coli* Spp. (8%) *Staphylococcus* spp. (62%) in RB. However it may be postulated that these anaerobic bacteria population might be correlated with the exposure of peripartum events and can interfere with the conception in RB cows with subclinical uterine infections without interrupting the normal cyclicality. However the specific role of these isolated bacteria and their exact mechanism with RB yet to clearly understand.

Chapter V
LIMITATION

A routine systematic collection and comprehensive analysis of reproductive data is key to determine if there is a repeat breeder problem and identification of the microbes may be detected for the specific causal agent if responsible. So, further extensive investigation should study on same topic to overcome the limitations of the current study and the real situation might be revealed.

Chapter VI

CONCLUSIONS

In conclusions, the study showed that RB syndrome in cows is a multifactorial problem. Age, peripartum events, academic qualification of owner, drainage system at farm, visual hygienic score of cows were identified as significant risk factors of repeat breeding syndrome.

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BIOGRAPHY

F. M. Yasir Hasib an intern veterinarian from Chittagong Veterinary and Animal Sciences University (CVASU) was born in Jamalpur, Bangladesh. He was born in a liberal and eminent family. After completing SSC and HSC from Nasirabad Govt. High School and Govt. Hazi Mohammad Mohsin College at 2009 and 2011 respectively, He was getting himself admitted into CVASU in 2013. He was intellectual and well-behaved person and a hard worker as well. He participate a number of activities as a student such as IVSA, Debating Society, AAVB, Animal welfare groups etc. and he will be a competent veterinarian in future.