

Bacterial contamination of ram semen used for artificial insemination in indigenous ewes

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Abstract

Ram semen was assessed for quality and presence of bacteria. Four ejaculates were collected from each of four rams twice a week using artificial vagina. The volume varied from 0.4 - 1.3 mL, colour from 2 - 4 (creamy to creamy-grey), mass activity from 3 - 5, sperm motility from 75 - 85%, viability from 80 - 95%, and concentration from, 2500 - 5000 × 10⁶/mL. The mass activity of ram R6 was significantly (P<0.05) higher (5.0 ± 0.0) compared with ram R1 (4.4 ± 0.5), R2 (3.9 ± 0.0) and R5 (4.7 ± 0.5). The mean motility was 81.7 ± 4.0, viability 90.0 ± 4.0 and concentration 3519.0 ± 545.6 × 10⁶/ml. *E. coli* and *Staphylococcus spp.* were found in all four rams' fresh semen confirmed by culture, staining and biochemical tests. However, *Bacillus spp.* was found only in ram R5. When the semen samples were treated with antibiotics there was no growth of bacteria after three days of incubation. It is suggested antibiotics control the transmission of microorganisms through AI in ewes. (*Bangl. vet.* 2017. Vol. 34, No. 1, 20 – 26)

Introduction

The sheep population in Bangladesh has increased by 2.5 times during the last decade with annual growth rate of 5% (BBS, 2008). The best rams can only be widely exploited through using artificial insemination (AI). However, AI can spread of infection through unhygienic insemination. Semen collection is not a sterile procedure, and some contamination with bacteria cannot be avoided (Aurich and Spergser, 2007; Bielanski, 2007). Semen may be contaminated with bacteria from the surface of the penis and prepuce, collection area, equipment and people. As a consequence, bacteria might contaminate the female's reproductive tract. To minimize these effects, antibiotics are included in ram semen extenders to prevent bacterial growth (Salamon and Maxwell, 2000). This study was designed to evaluate indigenous fresh ram semen and identify the microorganisms present in preserved semen used for AI.

Materials and Methods

Animal selection and management

Four indigenous rams 9 - 14 months old were used and maintained with balanced nutrition and hygienic conditions, dewormed and vaccinated routinely.

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Collection of semen

The semen was collected using artificial vagina and standard operating procedure (Marco *et al.*, 2005).

Evaluation of collected semen

The semen was evaluated for colour, volume, density, mass activity, motility, concentration and viability, using standard procedure. The samples having $\geq 70\%$ sperm motility, $\geq 85\%$ live percentages and $2500 \times 10^6/\text{mL}$ spermatozoa were only considered for semen dilution and preservation.

Extender preparation

Extender (tris, fructose, egg-yolk: TFE) was prepared according to Azizunnesa *et al.* (2016); (tris-base: tris 3.4g, fructose 0.5g, citric acid 2.0g, penicillin 100,000 IU, streptomycin 100 mg, 7% glycerol, deionized water 100 mL). The stock solution was stored at 4 to 5°C and, on the day of semen collection, the final extender was prepared by adding egg-yolk (10%). The semen was frozen and cryo-preserved using two step procedures.

Microbiological evaluation of semen

Isolation of bacteria

The semen samples (fresh and frozen) were inoculated into nutrient broth separately and incubated at 37°C overnight. The enriched broth was sub-cultured in nutrient agar, EMB agar, MS agar, and blood agar according to the method described by Cowan (1985).

Identification of the bacteria

For identification of bacteria, Gram's stain was performed according to the method described by Merchant and Packer (1967). Sugar fermentation test, MR-VP reaction, indole reaction, catalase test and coagulase test were performed.

Statistical analysis

All parameters were expressed as Mean \pm SD. The statistical analyses were done using SPSS 20.0 software program. One-way analysis of variance (ANOVA) was done to find out significant differences in reproductive parameters. One-way ANOVA: Post Hoc multiple comparisons (Duncan test) were done to find out significant differences in semen parameters.

Results and Discussion

Evaluation of semen

The parameters of semen following preservation are presented in Table 1. The sperm concentration of ram R6 was significantly higher compared with ram R1, R2 and R5.

The sperm motility of ram R6 and R5 were significantly higher compared with Ram R1 and R2. Also the sperm viability of ram R6 was significantly higher compared with ram R1, R2 and R5. Similarly, the mass activity of ram R6 was significantly higher compared with Ram R1, R2 and R5. There was no significant difference in semen volume and normal sperm morphology % between the rams. The volume of ejaculated semen varied from 0.4 - 1.3 mL. This agrees with a previous report (Moss *et al.*, 1988). Azizunnesa *et al.* (2014) stated that the normal ejaculated volume of semen of ram was 1.2 ± 0.0 mL. The colour of the ram semen was creamy to creamy-grey, graded as 2 - 4. This result agrees with the earlier reports of Bag *et al.*, 2002; Azizunnesa *et al.*, 2014. Bag *et al.* (2002) stated that the colour of ram semen varied from milky-white to pale cream. The sperm concentration of ram varied from 2500 to 5000×10^6 /mL, which agrees with the report of Mahmuda *et al.* (2015). Azizunnesa *et al.* (2014) and Mahmuda *et al.* (2015) stated that the sperm concentration of indigenous ram lay between 3900 to 4500×10^6 /mL. The mass activity varied from 3 - 5, which matches with the report of Cunha *et al.* (2012) who reported that the mass motility varied from 0 - 5 depending on species.

Table 1: Evaluation of fresh ram semen

Ram ID	Volume (mL)	Colour (1-4)	Mass activity	Sperm motility (%)	Viability (%)	Concentrations ($\times 10^6$ /mL)
R1	$0.8^a \pm 0.2$	$3.5^b \pm 0.6$	$4.4^b \pm 0.5$	$80.5^b \pm 4.3$	$89.3^b \pm 2.8$	$3496.7^b \pm 125.0$
R2	$0.7^a \pm 0.3$	$4.0^a \pm 0.0$	$3.9^c \pm 0.0$	$77.5^c \pm 2.7$	$85.0^c \pm 3.6$	$2795.4^c \pm 114.4$
R5	$1.0^a \pm 0.3$	$4.0^a \pm 0.0$	$4.7^b \pm 0.5$	$85.1^a \pm 4.3$	$92.5^a \pm 1.7$	$3663.2^b \pm 491.8$
R6	$0.9^a \pm 0.2$	$4.0^a \pm 0.0$	$5.0^a \pm 0.0$	$83.7^b \pm 4.7$	$93.3^a \pm 1.0$	$4120.5^a \pm 93.5$
Over all mean	0.8 ± 0.3	3.9 ± 0.3	4.5 ± 0.5	81.7 ± 4.0	90.0 ± 4.0	3519.0 ± 545.6

The mean values with different superscript within the same column differ significantly ($P < 0.05$)

Microbiological evaluation of fresh semen

Cultural properties of the isolates

All of the fresh semen collected from Ram1 (R1), Ram2 (R2), Ram5 (R5) and Ram6 (R6) were contaminated with *E. coli* and *Staphylococcus spp.* whereas, only one sample (R5) was contaminated with *Bacillus spp.* Bacteria were identified according to their colony characteristics on different culture media. *E. coli* produces greenish-black colonies with metallic sheen on EMB agar media (Fig. 1a). In MSA, *Staphylococcus* produces smooth, circular, small whitish colonies with change of media from red to bright yellow (Fig. 1b) whereas smooth, circular, small, whitish non-haemolytic colonies on blood agar media (Fig. 1c). In nutrient agar, *Bacillus* produces typical characteristics of medusa head type colony and grey haemolytic colonies on blood agar media (Fig. 1d, e). The colony properties of the isolated bacteria resemble the results of others such as Sohiddullah *et al.* (2016); Merchant and Packer (1967).

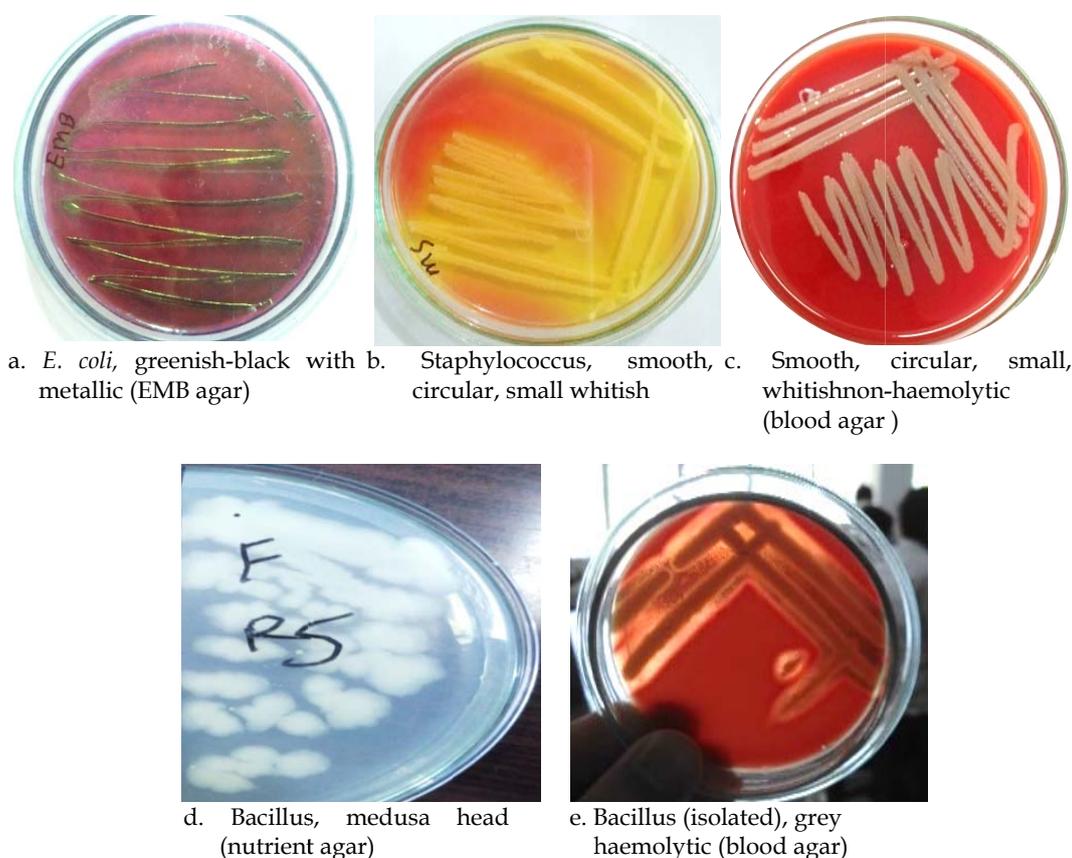


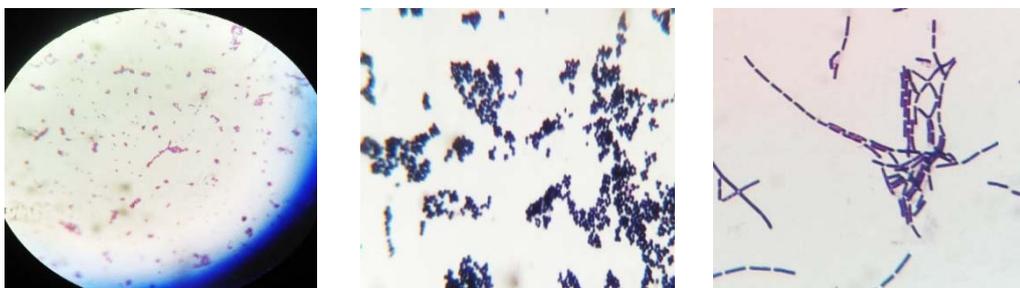
Fig. 1. Cultural - colonies characteristics of different bacteria from fresh semen samples

Morphological and staining properties of the isolated bacteria

E. coli showed single or paired short plump rods, gram negative staining property (Fig. 2a) whereas *Bacillus* showed single, large rod with violet coloured gram positive staining property (Fig. 2c). *Staphylococcus* showed cocci shaped grape-like clusters arrangement with gram-positive staining property (Fig. 2b). Thomas *et al.* (2005) reported that *E. coli* is Gram-negative, rod shaped, motile bacteria, which is similar to our findings. In *Staphylococcus spp.* the bacteria show Gram positive cocci shaped arranged in grapes like cluster, which matches with the earlier report of (Konuku *et al.*, 2012). Gram positive, violet coloured, large rod shaped organisms arranged in single colony in case of *Bacillus spp.* and this statement matches with the earlier report (Konuku *et al.*, 2012).

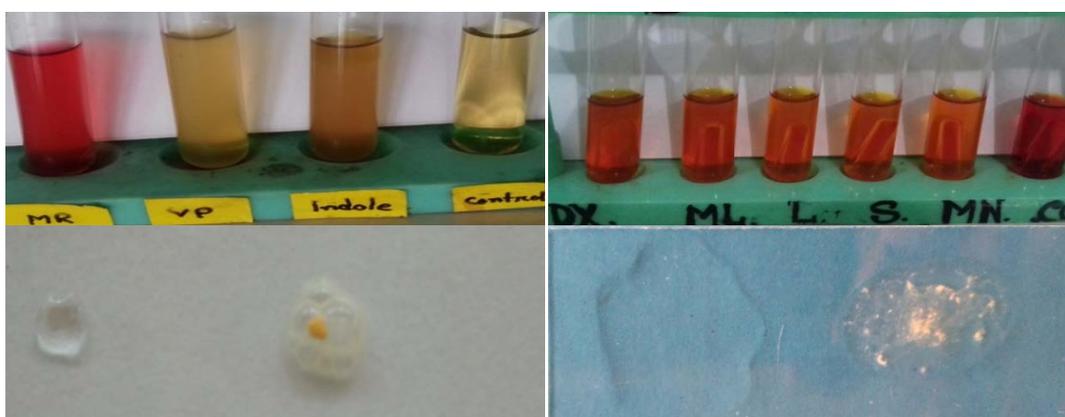
Biochemical test

In Biochemical tests, *E. coli* showed MR, Indole and VP test were positive and fermented dextrose, mannitol, lactose, maltose, and sucrose with acid production (Fig. 3a). The result supports the findings of Khaton *et al.* (2008); Sohidullah *et al.* (2016). In catalase test of *Staphylococcus spp.* showed bubble formation indicating positive reaction and in coagulase test curd formation indicates positive reaction (Fig. 3b). This statement matches with the earlier report of Kumar *et al.* (2011); Brook *et al.* (2002).



- a. *E. coli*, single or paired short plump rods (gram negative)
 b. *Staphylococcus*, cocci shaped grape-like clusters (gram-positive)
 c. *Bacillus*; single, large rod with violet coloured (gram-positive)

Fig. 2. Morphological and staining characteristics of the isolated bacteria



- a. *E. coli* in MR, Indole and VP test (positive and fermented dextrose, mannitol, lactose, maltose, and sucrose with acid production)
 b. *Staphylococcus spp.* catalase test (bubble formation, positive reaction and in coagulase test curd formation indicates positive reaction)

Fig. 3. Biochemical test of isolated bacteria

Microbiological evaluation of frozen semen

The semen samples treated with antibiotics showed no growth of bacteria after three days of inoculation (Fig. 4). This statement agrees with the report of Shin *et al.* (1988) who found that penicillin and streptomycin were active against the bacteria commonly found in the semen of rams such as *E. coli*, *Staphylococcus spp.* and *Bacillus spp.*



Fig. 4. Microbiological evaluation of frozen semen (no growth of bacteria)

Conclusions

We conclude that antibiotics in semen extender control the transmission of bacteria through AI.

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