Indian Journal of Animal Sciences **82** (6): 596–598, June 2012

Assessment of bacterial diversity in fresh bubaline semen

N RANA[1], R K VAID[2], S K PHULIA[3] and PAWAN SINGH[4]

Central Institute for Research on Buffaloes, Hisar, Haryana 125 001 India

Received: 8 Nov 2011; Accepted: 18 April 2012

Key words: Atypical, Bacteria, Buffalo bull, Semen

The contamination of fresh and preserved semen poses a great risk to successful breeding programme as it may lead to rapid decline in sperm motility (Roberts 1971). The microbes, because of their ubiquitous presence, have ample chance to contaminate semen during collection, processing and preservation. Artificial vagina, glasswares, semen extender, etc. are some of the common sources that may contribute to the bacterial load of semen during its processing (Bhakat *et al*. 1997). Bacterial presence in semen may impart resistance to the antibiotics used in semen extender. If not given due attention, the potential for transmission through AI would incresse. Trade in germplasm calls for the need for rapid, sensitive and specific diagnostic tests for semen free from pathogenic microbes (Eaglesome and Garcia 1992; Eaglesome *et al.* 1992). Bacterial presence in semen eventually elicits the production of reactive oxygen species (ROS) through macrophages and polymorphonuclear granulocytes as the first line of defence and resulting increased ROS generation impairs the functions of sperms and its fertilizing capacity (Aitken 1995). Thus, hygienic status of semen is important for vitality of spermatozoa and for the fertility of inseminated animals.

Fresh semen samples were collected from five Murrah buffalo bulls maintained under identical and optimal conditions of feeding and management. The bulls used for collection of semen were in sound reproductive health and free from any known specific diseases. Each sample of fresh liquid semen from bulls was collected through artificial vagina adopting the routine procedure. The samples were brought to the laboratory on ice and processed immediately. Two-fold serial dilutions of samples in 0.1 ml aliquots were prepared using 0.85% sterile normal saline solution (NSS) and inoculated by standard surface spread technique on 5% sheep blood agar and MacConkey agar plates (Quinn *et al*. 1994). The plates were incubated at 37°C for 48 h. Predominant morphologically distinct colonies were picked up at random from each inoculated plate. Pure bacterial cultures were obtained and further characterization and identification of the purified isolates was done as per the procedure of Cowan and Steel (2004) and Quinn *et al*. (1994). Briefly, Gram staining reaction, reaction on Hugh-Leifson medium, oxidase, catalase, urease, nitrate, reaction on nutrient agar and blood agar at 37°C under aerobic conditions, reaction on triple sugar iron agar, arginine, ornithine and lysine decarboxylase reaction and reaction to sugars like raffinose, trehalose, xylose, maltose, mannitol, arabinose and inositol were tested.

Identification of bacterial species was not intended to be exhaustive and only predominant colonies in each sample were isolated. A summary of the bacterial microflora isolated from bubaline semen is presented in Table 1. We identified three *Staphylococcus* spp., viz. *S. hyicus subs. chromogenes, S. auricularis* and *S. simulans* in the fresh semen. *Staphylococcus* spp., though have earlier been reported (Aleem *et al*. 1990, Gangadhar *et al*. 1986) in the literature, yet to the best of our knowledge, these 3 species have not been documented to occur in bovine or bubaline semen. *S. auricularis* has also been isolated from bovine meat in abattoir deboning rooms (Shale *et al*. 2005). *Staphylococcus chromogenes*, often implicated in bovine sub-clinical mastitis has been reported to be resident on haircoat, nares, vagina, teat skin and streak canal of heifers (White *et al*. 1989). *Staphylococcus simulans* too, usually implicated in bovine sub-clinical mastitis, is an opportunistic pathogen on human skin. A single isolate of *Enterobacter* sp. recovered in this study, and usually a cause of coliform mastitis in bovines and neonatal calf diarrohea, has also been reported by Angelo *et al*. (2006) in bovine semen.

Micrococcus spp. isolated in this study constitute normal

Present address: [1]Senior Scientist (nrana@scientist.com), Education Division, Indian Council of Agricultural Research, Krishi Anusandhan Bhavan-II, Pusa, New Delhi 110 012.

[2]Senior Scientist (rkvaid@yahoo.com), Veterinary Type Culture Centre, National Research Centre on Equines, Hisar 125 001, Haryana.

[3]Senior Scientist (sphulia@gmail.com), Central Institute for Research on Buffaloes, Hisar 125 001, Haryana,

[4]Principal Scientist (pawansingh@scientist.com, Artificial Breeding Research Centre, National Dairy Research Institute, Karnal 132 001

Table 1 Summary of bacterial flora isolated from semen

Bacterial isolate	Isolations	Biochemical characteristics	Reports on bovine semen
Micrococcus spp.	3	All resistant to furazolidone and showing atypical negative oxidase reaction, two isolates β-hemolytic	Frequently reported in bovine/ bubaline semen
Bacillus spp.	4	1 sensitive and 3 resistant to penicillin, 1 isolate α-hemolytic	Frequently reported in bovine/ bubaline semen
Atypical *Rhodococcus equi*	1	Atypical in being negative for NO_3 reduction	-
Moraxella bovis	1	β-hemolytic	Reported in frozen bovine semen (Gandhi *et al*. 2008)
Staphylococcus auricularis	1	Susceptible to furazolidone	-
Staphylococcus hyicus subs. chromogenes	1	Susceptible to furazolidone	-
Staphylococcus simulans	1	Resistant to furazolidone	-
Enterobacter spp.	1	No gas from glucose	Angelo *et al*. (2006)

skin flora of humans and other mammals. They are also found in soil, food products, air, etc. and may also cause bovine sub-clinical mastitis. *Bacillus* spp. that occur in a wide range of habitat are frequently reported to occur in bovine and bubaline semen. *Bacillus* spp., *Staphylococcus* spp. and *Micrococcus* spp. have previously been reported (Jasial *et al*. 2000a,b; Balakrishnan *et al*. 2006, Prabhakar *et al*. 1993) to occur in buffalo bull semen. Ramaswamy *et al*. (2002) and Singh *et al*. (2007) have also reported the prevalence of *Bacillus* spp. and *Staphylococcus* spp. in buffalo bull semen.

Moraxella bovis, a cause of Infectious bovine keratoconjunctivitis in bovines, and *Rhodococcus equi*, a cause of suppurative bronchopneumonia, lymphadenitis and ulcerative enteritis in foals were also recovered in the present study. Latter can multiply in soils enriched with equine feces and has also been reported in equine ejaculates (Ortega-Ferrusola *et al*. 2009). The species of *R. equi* identified in the study was atypical as it was biochemically not capable of reducing nitrate. Similar results were for the 3 species of *Micrococcus*, as they exhibited negative oxidase reaction.

It is noteworthy that some of the isolates have not been reported in the literature as microflora of semen. The presence of these bacteria is significant as they may compete with spermatozoa for nutrition, alter the physiological behaviour of the female reproductive tract leading to failure of fertilization, early embryonic death, abortion and/or repeat breeding.

SUMMARY

Fresh semen samples from Murrah buffalo bulls after dilution were streaked on 5% sheep blood agar and MacConkey Lactose Agar. Predominant morphologically distinct colonies growing on blood agar were picked up at random to isolate pure bacterial cultures. A total of 13 cultures (11 gram positive and 2 gram negative) were finally subjected to characterization. The bacteria identified belonged to atypical *Micrococcus* spp., *Bacillus* spp., atypical *Rhodococcus equi*, *Staphylococcus auricularis*, *Moraxella bovis*, *Staphylococcus chromogenes*, *Staphylococcus simulans* and *Enterobacter* spp. Three Staphylococcal spp. and *R. equi* isolated in this study are being reported for the first time in bubaline semen.

REFERENCES

Aitken R J. 1995. Free radicals, lipid peroxidation and sperm function. *Journal of Reproduction and Fertility* **7**: 659–68.

Aleem M, Chaudhry R A, Khan N U, Rizvi A R and Ahmed R. 1990. Occurrence of pathogenic bacteria in buffalo semen. *Buffalo Journal* **6**: 93–98.

Angelo M D, Pavão D L, Melo G M, Rojas N, Souza R J, Athayde C, Galuppo A G, Pinheiro E S. 2006. Acceptable microorganisms concentration in a semen sample for *in vitro* embryo production. *Brazilian Journal of Microbiology* **37**: 571–72.

Balakrishnan G, Saravanabava K and Dorairajan N. 2006. Microbial quality and antibiotic sensitivity pattern of buffalo frozen semen. *Indian Veterinary Journal* **83**: 1225–26.

Bhakat C, Raina V S and Nagpaul P K. 1997. Assessment of bacterial load in accessories used in artificial insemination. *Indian Journal of Animal Production and Management* **13**(4): 223–24.

Cowan S T and Steel K J. 2004. *Manual for the Identification of Medical Bacteria*. 3rd edn. Cambridge University Press, London.

Eaglesome M D and Garcia M M. 1992. Microbial agents associated with bovine genital tract infections and semen. Part I. *Brucella abortus*, *Leptospira, Campylobacter fetus* and *Trichomonas foetus*. *Veterinary Bulletin* **62**: 743–75.

Eaglesome M D, Garcia M M and Stewart R B. 1992. Microbial agents associated with bovine genital tract infection and semen. Part II. *Haemophilus somnus, Mycoplasma* spp. and *Ureaplasma* spp., *Chlamydia*; pathogens and semen contaminants; treatment of bull semen with antimicrobial agents. *Veterinary Bulletin* **62**: 887–910.

Gandhi A, Sharma A, Dhar P, Katoch V, Thakur A and Kumar R.2008. Isolation of *Moraxella bovis* from frozen bovine semen and determination of microbial load. *Indian Journal of Microbiology* **48**(3): 405–07.

Gangadhar K S, Rao A R, Krishnaswamy B and Rao S U. 1986.

Bacterial and fungal types and their load in the frozen semen of buffalo bulls. *Indian Veterinary Journal* **63**: 48–53.

Jasial S, Katoch R C, Chachara D and Mahajan A. 2000a. Evaluation of bacterial load in fresh ejaculates of bovine and buffalo bull semen in Himachal Pradesh. *Indian Journal of Animal Sciences* **70**(5): 465–67.

Jasial S, Katoch R C, Narang D and Mahajan A. 2000b. The drug resistant bacteria associated with frozen semen technology of cow and buffalo bulls. *Indian Journal of Animal Sciences* **70**(5): 488–89.

Ortega-Ferrusola C, Gonzalez-Fernandez L, Muriel A, Macias-Garcias B, Rodriguez-Martinez H, Tapia J A and Pena F J. 2009. Does the microbial flora in the ejaculate affect the feuzability of stallion sperm. *Reproduction in Domestic Animals*. **44**: 518–22.

Prabhakar T G, Premkumar D B, Paul W M, Rao C V and Venkatesan R A. 1993. Bacterial analysis in the frozen semen of buffalo bulls. *Indian Journal of Animal Sciences* **63**: 644–45.

Quinn P J, Carter M E, Markey B K and Carter G R. 1994. *Clinical Veterinary Microbiology*. pp. 209–36. Mosby Year Book Europe Ltd., London.

Ramaswamy V, Latha N, Gnanasubramanian T and Manickam R. 2002. Aerobic bacteria in buffalo semen and their antibiogram. *Indian Journal of Animal Reproduction* **23**(2): 117–19.

Roberts, S J. 1971. *Veterinary Obstetrics and Genital Disease*. Scientific Book Agency, Kolkata.

Shale K, Lues J F R, Venter P and Buys E M. 2005. The distribution of *Staphylococcus* sp. on bovine meat from abattoir deboning rooms. *Food Microbiology* **22** (5): 433–38.

Singh M P, Prasad J K, Saxena M S and Kumari P. 2007. *Indian Journal of Animal Reproduction* **28**(1): 30–33.

White D G, Harmon R J, Matos J E S and Langlois B E. 1989. Isolation and identification of coagulase-negative *Staphylococcus* species from bovine body sites and streak canals of nulliparous heifers. *Journal of Dairy Science* **72**(7): 1886–92.