International Journal of Livestock Research, Vol. 10 (7) July' 2020
http://www.ijlr.org
eISSN: 2277-1964
NAAS Rating 2020 – 5.36

,

Original Research

Estimation of Bacterial Load from Preputial Washing of Jaffrabadi Bulls

S. S. Parikh[1]*, F. S. Kavani[2], A. R. Bariya[3], A. S. Patel[3], R. B. Makwana[1], G. B. Solanki[1] and T. K. Patbandha[4]

[1]Assistant Professor, Cattle Breeding Farm, Junagadh Agricultural University, Junagadh, Gujarat, INDIA
[2]Retired Professor & Head, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, AAU, Anand, Gujarat, INDIA
[3]Assistant Professor, Department of Livestock Products Technology, College of Veterinary Science & Animal Husbandry, JAU, Junagadh, Gujarat, INDIA
[4]Assistant Professor, Livestock Production and Management, College of Veterinary Science & Animal Husbandry, JAU, Junagadh, Gujarat

*Corresponding Author: drss.parikh@gmail.com

How to cite this paper: Parikh, S., Kavani, F., Bariya, A., Patel, A., Makwana, R., Solanki, G., & Patbandha, T. (2020). Estimation of Bacterial Load from Preputial Washing of Jaffrabadi Bulls. *International Journal of Livestock Research*, 10(7), 41-46. doi: http://dx.doi.org/10.5455/ijlr.20200513115035

Received : May 13, 2020
Accepted : Jun 10, 2020
Published : Jul 31, 2020

Abstract

The present study was conducted to identify the bacterial load of preputial cavity in adult and young Jaffrabadi bulls which were selected from the Cattle Breeding Farm, JAU, Junagadh. Preputial wash material was collected by syringe method after infusing 125 ml of warmed normal saline into preputial cavity. Bacterial loads from samples were estimated by using standard plate count method. The bacterial loads in individual preputial samples of Jaffrabadi bulls were 168, 101, 189, 68, and 57 cfu/ml with overall mean of 116.60±26.50 cfu/ml. Majority of bulls had gram positive bacterial except one bull had gram negative bacterial count. Preputial cavity is the main source of contamination of ejaculated semen and bacterial load present in preputial cavity which cause the detrimental effects on the semen quality besides the fertility. So, these findings could be helpful for proper managemental interventions to augment the semen quality and future fertility by using a drug or reduce the load by serial preputial washings.

Keywords: Bacterial Load, Jaffrabadi Bulls, Preputial Wash, Standard Plate Count

Introduction

The preputial cavity of bull is likely a vital source of bacterial pathogens that lead to reproductive diseases and the risk of microbial spread during the collection of semen and thereafter use in artificial insemination (Balqui *et al.,* 2018). The success of AI programme mainly depends on quality semen production and proper AI practices (Patel *et al.,* 2011). Therefore, production of quality semen is the keen interest of each and every semen stations throughout the India (Meena *et al.,* 2015). Most of semen stations strictly follow minimum standard protocols for frozen semen production through disease testing at regular interval to avoid the spread of the microbial infections. One of the important factors, which influence semen quality (Diemer *et al.,* 1996) and further fertility (Griveau *et al.,* 1995) is a bacterial load in preputial cavity, therefore, it is considered in the quality control of semen (Martin *et al.,* 2010). High microbial load in preputial cavity is a reflection of unhygienic management practices in various steps of bull management (Rahmi *et al.,* 2015). Sheath washing and scraping are the two most widely used techniques for the collection of preputial material without interfering its effectiveness (Schonmann *et al.,* 1994).

Microbial contamination has an effect on motility, morphology and various semen quality parameters (Nazee *et al.,* 2012) which may be due to direct effect or competition for nutrients (Yaniz *et al.,* 2010) between aerobic and anaerobic bacteria in collected semen while some opportunistic pathogenic organisms may cause reproductive disorders in female animals expressed by lower conception rate, increase in embryonic mortality, abortion and other complications. Several types of bacteria have been isolated from frozen semen (Mozo-Martin *et al.,* 2010) which leads to the production of phagocytic cells (macrophages and PMNs) and these cells give rise to reactive oxygen species that compromise the sperm function and reduces its capacitation as well as fertilization capability (Morrell, 2006). Hence, this study was planned to access the preputial washings for presence of bacterial load prior to the semen collection from Jaffrabadi buffalo bulls.

Materials and Methods

Animals, Ration and Experimental Design

A total five healthy breeding Jaffrabadi buffalo bulls were selected from the Cattle Breeding Farm, JAU, Junagadh for the present study. Throughout the study period, animals were maintained under similar feeding (green fodder, hay, compounded concentrate and mineral mixture) and other farm practices under stall feeding system. All bulls were healthy and kept under strict control measures for internal and external parasitism, as they undergo a periodical deworming and prophylactic vaccination against the endemic diseases. Bulls were kept in the individual bull pen separated by solid partitions that restricted both direct physical and visual contact of bulls in adjacent pens as well as free movements within the shed. Bulls were exercised once a week, the day before semen collection in the rotary bull exerciser so as to maintain the sexual vigor of bulls and ensure quality semen production. Bulls were regularly screened for sexually transmitted disease and as per the farm schedule other herd-health monitoring program was followed to ensure good health.

Collection of Preputial Washing

Preputial washings were collected by syringe method which is one of the techniques to eliminate the bacterial load from Jaffrabadi bulls (Fig. A). Bulls were restrained in a sturdy crush with a neck clamp, so that the operator became safe and move easily in uncertain circumstances. Prior to preputial washing, the sheath was inserted into preputial orifice, massaged the preputial cavity to remove any air if any. The preputial washing was done by infusing 125 ml/bull sterile and warm normal saline solution (37°C) with the help of a sterile disposable plastic syringe (60 ml) and white plastic AI sheath. The sheath was introduced upto distance of 15-20 cm (6-8 inches) into preputial cavity, sealed the orifice by gripping above the sheath with the fingers of one hand and normal saline was injected into the cavity. With the free hand briskly massaged the fluid within the preputial cavity and gently withdraw the fluid back through the same sheath into the syringe (Fig. B). Finally, preputial flushing samples (Fig. C) were transferred to the laboratory for further processing within 6 hours.

Figure A: Jaffrabadi bulls used; **B:** Collection of preputial wash; **C:** Preputial wash samples; **D:** Pour plate method for bacterial load count; **E:** Colony count by quabac colony counter

Estimation of Bacterial Load by Pour Plate Method

The preputial washing samples were subjected to standard plate count using pour plate technique as per Cruickshank *et al.* (1975). Duplicate plates were prepared for each sample. The serial dilutions of the preputial wash were prepared with triple glass distilled water. Simultaneously, plate count agar also weighed, reconstituted in triple distilled water and autoclaved at 121°C for 20 minutes at 15lb pressure. The plate count agar was contained casein enzymichydrolysate – 5g/L, Yeast extract- 2.5 g/L, Dextrose – 1g/L, sodium chloride 6.5g/L and agar – 15g/L. A dilution of 10^{-1} (1: 10) to 10^{-3} (1000) prepared serially for bacterial load estimation from each preputial wash of bull. Each dilution of sample in duplicates was added to petri-plates and mixed with autoclaved plate count agar pre-warmed at 45ºC to the same plates. The plates were rocked gently for mixing of inoculum uniformly into the agar and allowed to solidify in the laminar air flow (Fig. D). The solidified plates were inverted and incubated at 37ºC for 24-48 hours to observe the formation of colony forming unit. All the colonies were counted including those that were embedded colonies that appear much smaller than those which happen to form on the surface with the help of Quebec colony counter. The average colony count was calculated from each plate (Fig. E). The bacterial count (cfu/ml) of each sample was counted by multiplying the dilution factor with number of colony in plate. The data of bacterial load of preputial washings were compiled, tabulated and analyzed by descriptive statistics for mean and standard error.

Results and Discussion

All the preputial washing samples were contained gram positive bacteria whereas, 11088 bull contains both gram positive and gram-negative bacteria (Table 1). The present study indicated that 3 bacterial types were isolated from both young and adult bulls, namely *Staphylococcus spp.*, *Corynebacterium spp.* and *E. Coli*.

Table 1: Bacterial load in preputial washings of five Jaffrabadi bulls estimated by SPC

S. No.	Bull Name /No.	Bacterial load cfu/ml in preputial flush (x 10^{-1} to 10^{-2})	Organisms observed
1	Chaman	168	*Staphylococcus spp., Corynebacterium spp.*
2	11086	101	*Staphylococcus spp., Corynebacterium spp.*
3	Badal	189	*Staphylococcus spp.*
4	11088	68	*E. coli, Staphylococcus spp.,*
5	Alok	57	*Corynebacterium spp.*
Overall		**116.60±26.50**	

It may be due to the organisms present at normal flora and it may come from the contact of outer orifice of prepuce with contaminated floor during the lie down of animals on the prepuce (Jansen and Wool-Board, 1983). The current study was demonstrated that the high ratio of *Staphylococcus* and *Corynebacterium spp*. bacteria from the prepuce of Jaffrabadi bulls, these results are compatible with study of Ling and Ruby (1978) who found that *Staphylococcus aureus* was the most unique aerobic microorganisms which secluded from the preputial cavity of normal adult male dogs. Mohamed *et al.* (2016) suggested that the *Staphylococcus aureus* was the most common member of normal microflora in sheep skin and it may be considered as the main etiology for high ratio of isolate *Staphylococcus aureus* from the prepuce in rams. Same results reported by Shallali *et al.* (2001) who isolated same bacteria from the vagina of healthy ewes and the *Staphylococcus aureus* was isolated in a highest ratio than other bacteria. They also suggested that the transmission of these bacteria occurred through the natural services. The presence of pathogenic bacteria in the prepuce of rams might be come from services ewes infected with metritis, endometritis, vaginitis, or urinary system infection, and these rams which have high ratio of contamination may spray the pathogenic bacteria to others females during natural cervices (Daher *et al.,* 2019). Balqis *et al.* (2018) reported presence of *E. fergusonii* bacterium in preputial swab samples of clinically healthy Aceh bulls and stated that the bacterium can be spread during natural mating or semen collection processing for artificial insemination.

In this study, we used 125 ml of warmed normal saline for washing of preputial cavity. Reports are also available regarding the use of volume for preputial washing fluid (100 ml- 200 ml) can be used for preputial washing without any specific recommendation (Kumar *et al.,* 2019). Variable number of bacterial counts has appeared in various reports pertaining to the volume of washing fluid. These variations of results suggest that, the volume of fluid (100-200 ml) which is being generally used for douching and cleaning of the prepuce (Bindra *et al.,* 1994), regardless of species is inadequate for proper washing because different bovine species or individual have different holding capacity of preputial sac (Kumar *et al.,* 2017). Average microbial load from preputial wash samples of Jaffrabadi bulls were assessed by standard plate count agar (SPC) method and presented in table-1. The estimated bacterial load (cfu/ml) in preputial washing of each bulls were 168, 101, 189, 68 and 57 with overall mean of 116.60±26.50. Several researchers reported wide variation of bacterial count (53 to 672.0 x10^{-3}cfu/ml) in preputial wash by 100 or 200 ml NSS or PBS prior to semen collection (Reddy *et al.,* 1971; Kher and Dholakia, 1984; Bindra *et al.,* 1994; Ahmed *et al.,* 2001; Meena *et al.,* 2015). The mean bacterial load of 3187.17, 2536.33 and 2292.83 (cfu/ml x 10^{-3}), respectively in preputial washings by saline, savlon and KMnO4, respectively (Meena *et al.,* 2015). The mean bacterial load observed in preputial washings of Jaffrabadi buffaloes is comparatively lower than Murrah buffalo bulls (Meena *et al.*, 2015). The variation of bacterial load in semen in different studies may be attributed to age, species, season, breed and types of prepuce (Reddy *et al.,* 1971; Kher and Dholakia, 1987; Sannat *et al.,* 2015).

The current study also revealed that the bacterial load was found to be more in adult than the young bulls. Same results were recorded by Wahid *et al.* (2003) and Navya, (2012) who found the lowest bacterial numbers in the youngest age groups, while the greatest numbers of bacteria were found in the oldest group and this may be attributed to the deeper epithelial crypts in the prepuce and penis of the older male.

Conclusion

In Jaffrabadi bulls, the bacterial load of preputial washing was observed 116.60±26.50 cfu/ml. Three types of bacteria such as, *Staphylococcus spp., Corynebacterium spp*. and *E. Coli* were isolated from both young and adult bulls. The bacterial presence in preputial washing may affect semen quality in Jaffrabadi bulls, so prior to semen

collection preputial washing should be practiced to minimize bacterial load.

Acknowledgements

The authors thank to Research Scientist & Head, CBF and Dean, College of Veterinary Science and Animal Husbandry for providing necessary facilities.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Ahmed, K., Kumar, A.A. and Mohan, G. (2001). Bacterial flora of preputial washing and semen of Murrah buffalo bulls and their antibiotic sensitivity pattern. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases*, *22*(1): 63-64.
2. Balqis, U., Hambal, M., Admi, M., Safika, Meutia, N., Sugito, Darsul, Abdullah, M.A.N., Ferasyi, T.R., Lubis, T.M., Abrar, M. and Darmawi. (2018). *Escherichia fergusonii* identified in preputial swabs from healthy Aceh cattle by phylogenetic 16S rRNA analysis. *Malaysian Journal of Microbiology*, *14*(3): 229-235.
3. Bindra, D.S., Pangawkar, G.R., Matharoo, J.S. and Singh, M. (1994). Effect of preputial washing on semen quality of buffalo bulls. *Indian Journal of Animal Reproduction, 15*: 75-76.
4. Cruickshank, R., Duguid, J.P., Marmoin, B.P. and Swain, R.H.A. (1975). *Medical Microbiology: The Practice of Medical Microbiology.* 12thedn, Vol. *2*, Churchil, Livingstone, Edinbourgh.
5. Daher, N.N., Ismaeel, M.A. andNoomi, B.S. (2019). Bacterial contamination of prepuce in young and adult rams in Salahaddin province. *Tikrit Journal for Agricultural Sciences. 18*(4): 120-123.
6. Diemer, T., Weidner, W., Michelmann, H.W., Schiefer, H.G., Rovan, E. and Mayer, F. (1996). Influence of *Escherichia coli* on motility parameters of human spermatozoa *in vitro*. *International Journal of Andrology, 19*(5): 271-277.
7. Griveau, J.F., Domount, E., Renard, P., Challegani, J.P. and Lelannou, D. (1995). Reactive oxygen species lipid peroxidation and enzymatic defense system in human spermatozoa. *Reproduction, 103*(1): 17-26.
8. Jansen, B. C. and Wool-Board, S. A. (1983). The epidemiology of bacterial infection of the genitalia in rams. *Journal of Veterinary Research, 50*: 275-282.
9. Kher, H.N. and Dholakia, P.M. (1984). Bacteriological studies of bovine semen. *Indian Journal of Animal Reproduction, 5*(1):78-84.
10. Kumar, A., Prasad, J. K., Ghosh, S. K., Das, G. K., Chakravarti, S., Yadav, H. K. and Verma, M.R. (2017). Effect of volume of flushing fluid on bacterial load in preputial wash of Murrah and Sahiwal bulls. *Ruminant Science, 6*(1): 167-170.
11. Kumar, A., Prasad, J.K., Ghosh, S.K., Das, G.K., Kumar, B., Yadav, H.C. and Katiyar, R. (2019). Effect of volume of washing fluid on bacterial load in preputial wash, fresh and post-thaw semen in crossbred bulls. *The Pharma Innovation Journal, 8*(9): 244-248.
12. Ling, G.V. and Ruby, A.L. (1978). Aerobic bacterial flora of the prepuce, urethra, and vagina of normal adult dogs. *American Journal of Veterinary Research, 39*(4): 695-698.
13. Martin, L.O.M., Munoz, E.C., De Cupere, F., Van Driessche, E., Echemenda-Blanco, D., Rodriguez, J.M. and Beeckmans, S. (2010). Bacterial contamination of boar semen affects the litter size. *Animal Reproduction Science, 120*(1-4): 95-104.
14. Meena, G.S., Raina, V.S., Gupta, A.K., Mohanty, T.K., Bhakat, M., Abdullah, M. and Bishist, R. (2015). Effect of preputial washing on bacterial load and preservability of semen in murrah buffalo bulls. *Veterinary World, 8*(6): 798-803.
15. Mohamed, H.A.A., Van Klink, Ed. G.M. and ElHassan, S.M. (2016). Damage caused by spoilage bacteria to the structure of cattle hides and sheep skins. *International Journal of Animal Health and Livestock Production Research, 2*(1):39-56.

16. Morrell, J.M. (2006). Update on semen technologies for animal breeding. *Reproduction in Domestic Animals, 41*(1): 63-67.
17. Mozo-Martín, R., Dahmani, Y., Larraz, C. and Ubeda J.L. (2010). Main bacterial species isolated from commercial seminal doses and antibiotic activity. *Magapor, 22*: 57-61.
18. Najee, H.B., Al-Shawii, A.M and Abd-Al Rahman, L.Y. (2012). Bacterial contamination of imported bulls frozen semen. *Al-Anbar Journal of Veterinary Sciences, 5*(1): 54-62.
19. Navya, M. (2012). Bacterial load in the neat, extended and frozen bull semen and its antibiogram. M.V.Sc. Thesis, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar.
20. Patel, H.V., Patel, R.K. and Chauhan, J.B. (2011). Biochemical properties of microbial load in frozen semen of cattle. *Wayamba Journal of Animal Science, 3*: 117-121.
21. Rahmi, Y., Darmawi, Abrar, M., Jamin, F., Fakhrurrazi and Fahrimal, Y. (2015). Identification of *Staphylococcus aureus* in preputium and vagina of horses (*Equuscaballus*). *Journal Medica Veterinaria, 9*(2): 154-158.
22. Reddy, B.J.C., Krishnamurthy, P.S. and Venkataswami, V. (1971). Bacterial flora of prepuce and the effect of intra-preputial treatment on the bacteriological quality of semen. *Indian Veterinary Journal, 48*:722-727.
23. Sannat, C., Nair, A., Sahu, S.B., Sahasrabudhe, S.A., Kumar, A., Gupta, A.K. and Shende, R.K. (2015). Effect of species, breed and age on bacterial load in bovine and bubaline semen. *Veterinary World, 8*(4): 461-466.
24. Schonmann, M.J., BonDurant, R.H., Gardner, I.A., Hoosear, K.V., Baltzer, W., Kachulis, C.,Van- Hoosear, K.(1994). Comparison of sampling and culture methods for the diagnosis of *Tritrichomonas foetus* infection in bulls. *The Veterinary Record, 134*(24): 620-622.
25. Shallali, A.A., Hussein, A.M., Salih, M.M. and Dafalla, E.A. (2001). A preliminary report on bacteria isolated from the female genital tract of Sudanese sheep and goats. *The Sudan Journal of Veterinary Research, 17*: 56-63.
26. Wahid, Z., Hussen, T. and Abdul Wadood, E. (2003). Bacterial causes of endometritis in cattle. *Basrah Journal of Veterinary Research, 1*(1, 2): 23-32.
27. Yaniz, J.L., Marco-Aguado, M.A., Mateos, J.A. and Santolaria, P. (2010). Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15°C. *Animal Reproduction Science, 122*(1-2): 142-149.

****