



**ASSESSMENT OF GENITAL INFECTION AGENTS IN BULLS OF DAIRY FARMS IN
EAST AZERBAIJAN PROVINCE, IRAN**

KHODAVANDPOUR A^{1*} AND MOSAFERI S²

1: Student of Veterinary Medicine, Collage of Veterinary Medicine, Tabriz Branch, Islamic

Azad University, Tabriz, Iran

2: Department of Clinical Science, Collage of Veterinary Medicine, Tabriz Branch, Islamic Azad

University, Tabriz, Iran

ABSTRACT

The most important infectious diseases that affect fertility of the bull, and their transmission via semen are reviewed in this article. Present study was carried out in 10 dairy farms of east Azerbaijan province, Iran. This study was comprised of 100 bulls in which 20 of them were selected by chance. In this study, after injection of Xylazine at a dose of 0.25 ml/100khBW, sampling was done from tail vein and direct swabbing from intrapreputial area. For this mean, blood sampling was done using venoject from tail vein and swabbing was done from intrapreputial area without crashing to extra area then swabs were transferred into the normal saline in order to examination of Trichomonas, campylobacter and haemophilus. Also, sampled bloods were examined in order to existence of antigen against BVD, antibody against IBR, Leucosis and Leptospirosis using ELISA method. Results showed that Trichomonas fetus, Campylobacter fetus, Haemophilus and BVD was not detected in samples. But, IBR was positive in all of the samples obtained from bulls. Of 20 samples, 3 of them (15%) were positive in term of leucosis. Leptospirosis was negative in all samples.

Keywords: Transmissible Disease, Trichomonas, Campylobacter, Haemophilus, BVD, IBR, Leptospirosis, Infection, Bull

INTRODUCTION

Bovine reproduction in the developing countries continues to depend on a high percentage of natural service (mounting). It has been estimated that 85% of calves born in these countries come from natural service programmes [1]. Selecting a bull thus becomes a critical element leading to serious economic consequences if a particular bull has problems regarding infertility or is a disease transmitter [2]. Natural service continues to prevail, especially in beef cattle, since artificial insemination (AI) has been slow in developing [3]. This has meant that the situation has become worsened as semen is usually chosen without regard for technical criteria supported by genetic improvement, production or profitability impact studies [4]. Traditional bull selection has been based on morphological characteristics and their growth performance at determined ages more than an evaluation of their semen production and reproductive potential [5]. Specific exams are made on rare occasions for infectious diseases affecting the reproductive organs and which are vehiculated by semen. Just as a cow's fertility may be affected by a large number of infectious agents, the bull is exposed to the very same specific agents and many others directly affecting reproductive activity. This article was aimed

at reviewing current information concerning the main infectious agents affecting cattle fertility and their potential transmission through semen.

MATERIALS AND METHODS

Present study was carried out in 10 dairy farms of east Azerbaijan province, Iran. This study was comprised of 100 bulls in which 20 of them were selected by chance. In this study, after injection of Xylazine at a dose of 0.25 ml/100kgBW, sampling was done from tail vein and direct swapping from intrapreputial area. For this mean, blood sampling was done using venoject from tail vein and swapping was done from intrapreputial area without crashing to extra area then swaps were transferred into the normal saline in order to examination of *Trichomonas*, *Campylobacter* and *Haemophilus*.

Also, sampled bloods were examined in order to existence of antigen against BVD, antibody against IBR, Leucosis and Leptospirosis using ELISA method.

Swaps were examined using light microscopes using direct microscopic examination method.

BLV-Ab, *Leptospira interrogans* serovar hardjo-Ab, IBR-Ab and BVD-Ag were examined

using ELISA method and cybiotics, prionices, idvet and idexx kits, respectively.

Haemophilus was assayed using culture in chocolate agar and Trichomonas and campylobacter were assayed using modified zilnilson staining and light microscope.

RESULTS

Results showed that however Trichomonas fetus is one of the most important and common agent of reproduction disorder in all over the world was not detected in samples. As well as, Campylobacter fetus, as an important agent of abortion in cows and ewes, was not recognized. Haemophilus and BVD also were negative in our study.

But, IBR was positive in all of the samples obtained from bulls. So that, standard Ab titr assigned for IBR is range $<45-55<+$ but in our study was 93.3 and 100% of samples obtained was positive. Of 20 samples, 3 of them (15%) were positive in term of leucosis. Standard titr for leucosis is $<50<+$ so that in our study Ab mean titr was 23.05 and 55 in negative and positive samples, respectively. Leptospirosis was negative in all samples. Standard titr for Leptospirosis is $<20-45<+$ so that in our study Ab mean titr was 9.6 in all negative bulls.

Table 1: Direct Microscope Examination

Bull No.	Haemophilus Culture	Direct Macroscopic Examination For Trichomonas	Direct Microscopic Examination For Campylobacter
1089	N	N	N
1160	N	N	N
1099	N	N	N
777	N	N	N
977	N	N	N
1172	N	N	N
1137	N	N	N
1037	N	N	N
1335	N	N	N
1674	N	N	N
1653	N	N	N
1316	N	N	N
1235	N	N	N
1149	N	N	N
1000	N	N	N
1154	N	N	N
1453	N	N	N
1308	N	N	N
1304	N	N	N
1158	N	N	N

Table 2: ELISA Examination

Cow No.	BLV-Ab (pp)	IBR-Ab (pp)	Leptospira interrogans serovar Hardjo- Ab (pp)	BVDV-Ag
1099	55	93	8	N
1037	26	98	13	N
977	19	88	7	N
1089	22	92	12	N
1137	40	97	11	N
1160	58	95	12	N
1172	33	89	7	N
777	52	96	9	N
1000	19	93	8	N
1335	24	95	7	N
1653	17	92	7	N
1158	21	91	10	N
1235	18	97	11	N
1308	22	90	9	N
1149	19	93	10	N
1674	20	89	9	N
1316	23	93	10	N
1154	26	94	11	N
1453	25	96	9	N
1304	18	95	12	N
Normal Range	-<50<+	-<45-55<+	-<20-45<+	-

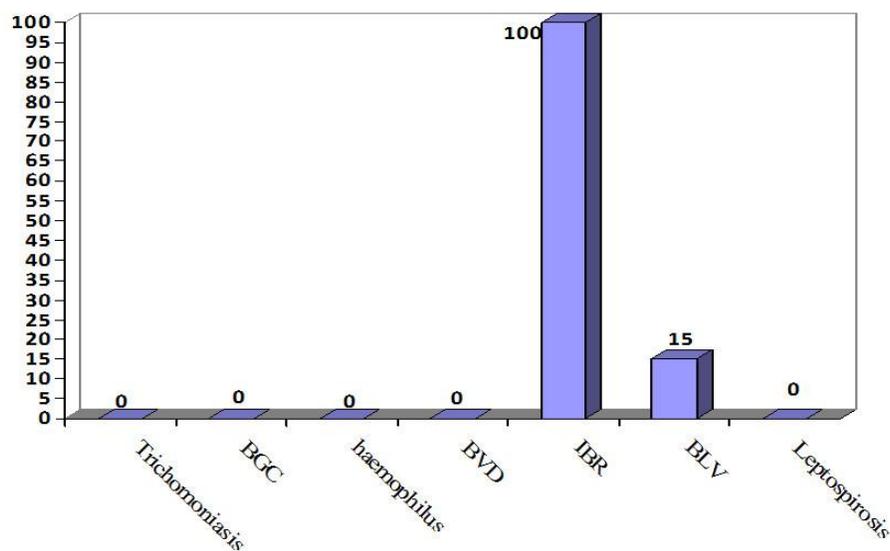


Figure 1: Diagram of Infectious Agents Comparison

DISCUSSION AND CONCLUSION

Saprophytic micro flora and other pathogens are found in bulls' preputial sac. Other infectious agents may be acquired through

venereal, respiratory or digestive infection from infected animals.

Bull fertility may be temporarily or permanently affected, depending on the type

of infectious agent (virus, fungi, bacteria, and protozoan) and the lesions produced on reproductive tract organs.

A large number of microorganisms have been isolated from semen and the prepuce. Twenty-seven different types of bacteria, fungi and blastomycetes were identified in 337 semen samples in a 1985 study and almost identical flora in 139 preputial washing liquids [6]; this means that there is controversy concerning the true effects of such agents on freezing, fertilizing power and the appearance of inflammatory processes [7].

The direct effect of pathogenic agents has been mainly focused on the testicles and glands forming part of the reproductive tract. Infection could be limited to a single organ (seminal vesicles) or spread extensively to other organs such as the epididymis, seminiferous ampoules, prostate, bulbourethral glands and urethra; in other cases they could reach the urinary bladder, urethra and kidneys [8].

The inflammatory processes producing these infections are complex and difficult to differentiate amongst the affected organs; they have thus been brought together under the term seminal vesiculitis syndrome [8]. Vesiculitis has ranged from 0.85 - 10% in studies evaluating the potential of young bulls' reproductive health [9]; however, greater

incidence has been found in slaughterhouses (49%) [10], significantly increasing their rejection rate.

Campylobacteriosis is a venereal disease affecting both animals and humans; it is produced by curved, gram-negative microaerophilic bacteria [11]. Two subspecies (*C. fetus* ssp. *venerealis* and *C. fetus* ssp. *Fetus*) are known which are highly related at genome level; however, they differ regarding the disease which they produce, the habitats they occupy and their biochemical characteristics [12]. Infection with *C. fetus* ssp. *venerealis* in cows is characterized by infertility, embryo death and abortion. The bacteria become located in the epithelium of a bull's penis, prepuce and urethra where chronic infection, lacking any characteristic sign, becomes established [13].

This disease, together with trichomoniasis, has the greatest importance in the transmission of disease through semen [14]. Bulls marked for AI must be declared free of such diseases even though adding antibiotics to semen leads to this pathogen being easily controlled [15].

The aetiological agent of Leptospirosis is a spirochete. The genus *Leptospira* includes two species: pathogenic and saprophytic. The pathogenic leptospire include 13 species and more than 260 serovars [16]. The leptospire

affecting cattle are mainly caused by the serovar hardjo making cattle a maintenance host for this serovar; in turn, two serologically indistinguishable but genetically different genospecies belong to it: *Leptospira interrogans* serovar hardjo (type hardjo-prajitno) and *Leptospira borgpetersenii* serovar hardjo (type hardjo-bovis). The serovar type hardjo-bovis is the most common in cattle around the world, whilst the hardjo-prajitno type has mainly been isolated from cattle in the United Kingdom [17].

A bull may present orchitis during the acute phase of leptospirosis, even though persistent infections are not very frequent and do not lead to the elimination of leptospires in semen [18]. By contrast, other researchers include leptospires within the group of infectious agents' vehiculated by semen as they survive at freezing and cryoconservation temperatures [19].

Infectious bovine rhinotracheitis (IBR) is a respiratory disease which is produced by bovine herpesvirus, type 1 (BHV-1), belonging to the Herpesviridae family. According to genomic and antigenic analysis, BHV-1 is divided into BHV-1.1 and BHV-1.2, in turn being subdivided into subtype BVH-1.2 and BHV-1.2b [20]. When BHV-1 affects the genital tract of cattle it causes infectious

pustular vulvovaginitis/ infectious pustular balanopostitis [21]. BHV-1 may also cause conjunctivitis, reproductive disorders and neonatal mortality [22, 23].

Bulls affected during an outbreak of the disease which occurred in an AI centre in Belgium presented brief pyrexia, uni- or bilateral orchitis and azoospermia. Mononuclear infiltration of the connective tissue, without neutrophils and degeneration of the germinal epithelium was found in one of the testicles examined by histopathology; the attempt at isolation led to positive results [24]. IBR-infected bulls eliminate the virus in semen during their whole lives [25], even though it has been thought that the virus cannot be eliminated from seropositive bulls if they are managed with low levels of stress [19]. The presence of the virus was detected in the post-nuclear region of the sperm's cephalic hood in a bull from a farm having fertility problems [26].

The pertinent worldwide literature is abundant regarding recognising this virus' transmission through semen or embryos [27-29]. Sanitary legislation thus establishes severe restrictions on importing biological material from countries where the disease is prevalent [30]. More recently, the World Organization for Animal Health (OIE) has included sanitary policy regarding this virus (as well as other

pathogens) in its guidelines concerning the taking of bovine semen, its treatment and recollecting and manipulating cattle embryos.

Bovine viral diarrhoea refers to a group of RNA virus classified within the pestivirus genus, 2 species being known: BVDV1 and BVDV2 [31]. The presence of BVDV2 is currently unknown in Colombia [32]. The necropsy findings and serological tests confirmed this case by revealing the presence of the disease of the mucosa in infected animals [33].

The protozoan parasite *Trichomonas foetus* is the aetiological agent of this venereal disease; three varieties have been described to date: Belfast, Brisbane and Manley [34]. The infection may occur asymptotically; however, some reports have associated this condition with transient balanopostitis [35].

Recent studies have been carried out involving the breeding animal as an important source of disease transmission via the coital route or the use of contaminated semen. [36] isolated *Trichomonas foetus* and *Campylobacter fetus* in 13.7% and 15% of bulls, respectively, in an investigation carried out on 103 farms in Colombia's 8 main cattle-raising regions. Eight of the 23 bulls had positive titres for *L. hardjo* and *L. pomonaseros*. A sanitary evaluation of 48 bulls from the Cundinamarca department

found 23.9% positivity for *Trichomonas*, 17.3% for *Campylobacter*, 43.4% for *Salmonella*, 28.2% for *Brucella* and 52.17% for *Leptospira* [37]. A 67.6% IBR prevalence has been reported inbreeding bulls in animals from Urabá in the Antioquia department [38]. A 15.3% seropositivity for IBR, 83% for BVD, 42% for bovine leucosis virus (BLV) and 92% for *Leptospira* spp was found in 11 dairy breed bulls from the savannah around Bogotá; reactorsto *Leptospira* serovars were pomona (62%), canicola (38%), hardjo (23%), gryphotyphosa and icterohaemorrhagiae (18%). The same study revealed the presence of IBR/BVD (17%), BVD/*Leptospira* spp (83%), BVD/BLV (42%), BLV/*Leptospira* spp (31%) and BVD/BLV/*Leptospira* spp infection (33%) [39]. This would mean that several infectious agents could converge on the same farm and in the same animal without the epidemiological importance and the dynamics of the different co infections being known regarding spermatid quality. An overall 37.4% seropositivity was found in 4, 230 samples received from different Colombian departments for IBR diagnosis by ELISA test. The sera having the greatest seropositivity came from the Santander and Cesar departments (72%) whilst those having the lowest seropositivity came from the upper

Magdalena valley (58.4%). An interesting observation concerned the bulls' high seropositivity (more than 60%) [40].

Many infectious agents may affect bull fertility. However, transmission through semen will depend on the donors' sanitary state. As shown in present study, contamination rate of genital infectious diseases is different among countries and also cities of a country which is vary because of management, environmental and ecological status and also number of animals in a farm. So, veterinarians of different areas should consider these factors in attempt to treatment and prevention of animals.

REFERENCES

- [1] Galina CS and Arthur GH, Review of cattle reproduction in the tropics: Part 6, The male, *Anim. Breed Abstr.*, 59, 1991, 403-412.
- [2] Galina CS, Horn MM and Molina R, Reproductive behavior in bulls raised under tropical and subtropical conditions, *Hormones and Behavior*, 52, 2007, 26-31.
- [3] Sabogal R and Obando H, Caracterización del material seminal bovino. Instituto colombiano agropecuario ICA subgerencia de prevención y control, división de

insumos pecuarios, Santa Fé de Bogotá, D.C, 2000, 7-37pp.

- [4] Giraldo JJ, Una Mirada al uso de la inseminación artificial en bovinos, *Revista Lasallista de investigación*, 4, 2007, 51-57.
- [5] Moraes JCF, Predição da fertilidade de touros empregados em monta natural. Congresso Brasileiro de Reprodução Animal. Belo Horizonte, Vol. 11. Colégio Brasileiro de Reprodução Animal, Belo Horizonte, 1995, 287.
- [6] Flastscher J and Holzmann A, Genital diseases in bulls: Importance for artificial insemination control measures, Inform e presentado a la 53a Sesión General de la OIE, 1985.
- [7] Parez M, Les plus importantes maladies genitales des bovins (prophylaxie, traitement, hygiène de la collecte du sperme) 11a Conf, Comisión Regional de la O.I.E. para Europa, O.I.E., Paris, 175-203, 1984.
- [8] McCauley AD, Seminal Vesiculitis in Bulls, In: *Current Therapy in Theriogenol.*, W.B. Saunders, London. 1980. 401-405.
- [9] Cavalieri J, Van Camp SD. Bovine seminal vesiculitis, A review and

- update, *Vet. Clin. North Am. Food Anim. Pract.*, 13, 1997, 233-241.
- [10] Ball L, Young S and Carroll EJ, Seminal vesiculitis syndrome, lesions in genital organs of young bulls, *Am. J. Vet. Res.*, 29, 1968, 1173-1184.
- [11] Skirrow MB, Diseases due to Campylobacter, Helicobacter and related bacteria. *J Comp Pathol* 1994; 111:113-149.
- [12] Brooks BW, Devenish J, Lutze-Wallace CL, Milnes D, Robertson RH and Berlie-Surujballi G, Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of Campylobacter fetus in bovine preputial washing and vaginal mucus samples, *Vet. Microbiol.*, 10, 2004, 377-384.
- [13] Eaglesome M and García M, Microbial agents associated with bovine genital tract infections and semen. Part I. *Brucella abortus*, *Leptospira*, *Campylobacter fetus* and *Tritrichomonas foetus*, *Vet. Bull.*, 62, 1992, 743-75.
- [14] Rovay H, Barth AD, Chirino-Trejo M and Martínez MF, Update on treatment of vesiculitis in bulls, *Theriogenol.*, 70, 2008, 495-503.
- [15] Thibier M. and Guerin B. Hygienic aspects of storage and use of semen for artificial insemination, *Anim. Reprod. Sci.*, 62, 2000, 233-251.
- [16] Adler B, Moctezuma A, *Leptospira* and leptospirosis, *Vet. Microbiol.*, 140, 2010, 287-296.
- [17] Grooms DL, Reproductive losses caused by bovine viral diarrhoea virus and leptospirosis, *Theriogenol.*, 66, 2006, 624-628.
- [18] Ellis WA, Cassels JA and Doyle J, Genital leptospirosis in bulls, *Vet. Rec.*, 118, 1986, 333.
- [19] Eaglesome MD and García MM, Disease risks to animal health from artificial insemination with bovine semen, *Rev. Sci. Tech. Off Int Epiz.*, 16, 1997, 215-225.
- [20] Barr BC, BonDurant RH, *Viral Diseases of the fetus*, In: Youngquist RS, Editor, *Bovine theriogenology*, Philadelphia: W.B. Saunders Company, 2000, 373-381.
- [21] Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA, 2004, *Virus Taxonomy, The Eighth Report*, Academic Press, San Diego, 1162.
- [22] Straub OC, BHV-1 Infectious bovine rhinotracheitis virus. In: Dinter Z, Morein B. (Eds.), *Virus Infectious of*

- Ruminants. Elsevier, Amsterdam, 1990, pp. 71-108.
- [23] Takiuchi E, Médiçi KC, Alfieri AF and Alfieri AA, Bovineherpesvirus type 1 abortions detected by a semi-nested PCR inBrazilian cattle herds, Res. Vet. Sci., 79, 2005, 85-88.
- [24] Thiry E, Pastoret PP, Dessy-Doize C and Hansen C, Herpesvirus in infertile bulls testicle, Vet. Rec., 108, 1981, 426.
- [25] Van Oirschot JT, Bovine herpesvirus 1 in semen of bulls and therisk of transmission: a brief review, Vet. Q., 17, 1995, 29-33.
- [26] Elashary MASY, Lamothe P, Silim A, Roy RS, Bovine herpesvirus 1 in the sperm of the bull from a herd with fertility problems, Can. Vet. J., 21, 1980, 336-339.
- [27] Bitsh V, Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection, Appl. Microbiol., 26, 1973, 337-343.
- [28] Kahrs RF, Effects of infectious bovine rhinotracheitis onreproduction, In: Current therapy in Theriogenology, W.B. Saunders, London, 1980, 250-254.
- [29] Kahrs RF and Littell RC, Detection of viruses in bovine semen (Influence of preparative centrifugation on isolation of IBRvirus).Amer Assoc. Veterinary Laboratory Diagnosticians, 23rd Ann. Proc, 1980, 251-262.
- [30] Hare WCD, Infectious diseases transmission by semen, In:Disease transmissible by semen and embryo transfer techniques, Technical series No 4 Office International Epizooties, 1982.
- [31] Ridpath JF, Bovine Viral Diarrhea Virus: Global Status, Vet. Clin. North Am. Food Anim. Pract., 26, 2010, 105-121.
- [32] Vargas DS, Jaime J and Vera VJ, Perspectivas para el control delVirus de la Diarrea Viral Bovina (BVDV) Rev. Colomb. Cienc. Pecu., 22, 2009, 677-688.
- [33] Borda A, Diarrea viral bovina en terneros y ternerasprocedentesde Holanda.Tesis de pregrado, Facultad de MedicinaVeterinaria Universidad Nacional de Colombia, Bogotá, 1975.
- [34] Skirrow SZ and Bondurant RH, Trichomoniasis, Vet. Bulletin, 58, 1988, 591-601.

- [35] Jubb KVR, Kennedy PC and Palmer N, Pathology of domestic animals, 3rd Ed., 3, 339-367. Academic Press, Orlando, 1985.
- [36] Griffiths IB, Gallego MI, De Leon LS, Levels of some reproductive diseases in the dairy cattle of Colombia, Trop. Anim. Filth Prod., 16, 1984, 219-223.
- [37] Villalobos R, Rozo J, Gallego MI. *et al.* Evaluación sanitaria en toros de fomento del Departamento de Cundinamarca. I. Estudios Bacteriológicos, Revista. Acovez, 36, 1986, 7-17.
- [38] Zuñiga I, Ossa JE and Hincapie O, Prevalencia de la Rinotraqueitis infecciosa bovina en reproductores de Urabá Antioquia para 1977, Rev. Colom. CiencPec., 2, 1978, 135-148.
- [39] Góngora A, Villamil L, Vera V, Parra J, Ramírez G and López G, Aislamiento de un Herpes Virus Bovinotipo 1 de secreción nasal esmegmaprepucial en un toro reproductor, Rev. Med. Vet. Zoot., 43, 1995.
- [40] Cotrino V, Anotaciones sobre Rinotraqueitis Bovina Infecciosa (IBR) como problema infeccioso que afecta la reproducción de bovinos, LMV, Bogotá, 1977.