

PCR DETECTION OF GENITAL INFECTIONS IN BULL SEMEN FROM DIFFERENT REGIONS OF UKRAINE, 2013 - 2016

Magomet Magomedovych Isakov^{1*}, Oleksii Serhiyovych Solodiankin¹,
Vitaliy Ihorovych Bolotin¹, Anton Pavlovych Gerilovych¹,
Aleksandar Potkonjak²

¹National Scientific Center „Institute Of Experimental and
Clinical Veterinary Medicine”, Kharkiv, Ukraine

²University of Novi Sad, Faculty of Agriculture, Department
of Veterinary Medicine, Novi Sad, Serbia

Abstract

The most important infectious diseases that affect fertility of the bull via semen are reviewed in this article. The article describes the results of a study of cryopreserved semen samples from different regions of Ukraine for the period 2013 to 2016. The strategy of regular testing of semen donors under official veterinary supervision has been adopted by governments worldwide as a means of avoiding the spread of pathogens and reducing excessive contamination of semen by ubiquitous bacteria and viruses. During this period, four hundred fifty (n=450) bull semen samples from 10 farms across Ukraine have been tested, PCR diagnostics was performed by the standard method developed in NSC «IECVM». According to the PCR analysis of cryopreserved semen from the bull-sires, 19 samples (6%) from Kharkiv region contained genetic material of *Mycoplasma spp.*, 2 samples from Poltava region (3.5%) were positive for BoHV-1, and 2 samples from Cherkasy region (4.5%) contained genetic material of *Chlamydia spp.* Based on the obtained results we can conclude that it is necessary to permanently perform PCR screening of bulls semen.

Key words: genital infections, *Mycoplasma*, BoHV-1, *Chlamydia*, semen, bulls, Ukraine

^{1*} Corresponding author: E-mail address: magomedisakovm@gmail.com

PCR DETEKCIJA GENITALNIH INFEKCIJA U SEMENU BIKOVA IZ RAZLIČITIH REGIONA UKRAJINE, 2013 - 2016

Magomet Magomedovych Isakov¹, Oleksii Serhiyovych Solodiantkin¹,
Vitaliy Ihorovych Bolotin¹, Anton Pavlovych Gerilovych¹,
Aleksandar Potkonjak²

¹Nacionalni naučni centar „Institut za eksperimentalnu i
kliničku veterinarsku medicinu”, Harkov, Ukrajina

²Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman
za veterinarsku medicinu, Novi Sad, Srbija

Kratak sadržaj

Najznačajnije infektivne bolesti koje utiču na plodnost bikova, a prenose se semenom, su opisane u ovom radu. Ovaj rad prikazuje rezultate istraživanja na zamrznutim uzorcima semena iz različitih regiona Ukrajine u periodu od 2013. do 2016. godine. Redovno testiranje semena bikova donora pod službenim veterinarskim nadzorom prihvaćeno je od strane vlada širom sveta kao način da se izbegne širenje uzročnika oboljenja i smanji kontaminacija semena ubikvitarnim bakterijama i virusima. Tokom ovog perioda testirano je četrstopedeset (n=450) uzoraka semena bikova sa 10 farmi širom Ukrajine, a PCR dijagnostika je izvršena standardnim metodom ravijenim u NNC „IEKVM”. Rezultati ispitivanja zamrznutih uzoraka semena priplodnih bikova pokazuju da su u 19 uzoraka (6%) iz Harkovske oblasti dokazani geni *Mycoplasma* spp., u dva uzorka iz Poltavske oblasti (3,5%) su dokazani geni BoHV-1, dok su dva uzorka iz Čerkaške oblasti (4,5%) bila pozitivna na *Chlamydia* vrste. Na osnovu ovih rezultata možemo zaključiti da je neophodno permanentno sprovoditi PCR skrining semena bikova na prisustvo patogenih uzročnika.

Ključne reči: polno prenosive infekcije, *Mycoplasma*, BoHV-1, *Chlamydia*, seme, bikovi, Ukrajina

INTRODUCTION

The main goal of artificial insemination (AI) in cattle is to achieve genetic improvement. However, transmission of infectious diseases by semen (sexual transmission, ST) constitutes a risk that must be avoided. The semen used for

AI must, therefore, be free of infectious agents (Wentink et al., 2000). The bull semen also plays a key role in reproductive excrete in cow herds, acting as a source of infectious diseases. The regular testing of semen donors under official veterinary supervision has been adopted by governments worldwide as a means of avoiding the spread of pathogens and reducing excessive contamination of semen by ubiquitous bacteria and viruses (Eaglesome et al., 1997).

Abortion among dairy cows is one of the sources of substantial economic losses in cattle industry. Although the risk of abortion depends on several factors (i.e., genetic abnormalities, heat stress, toxic agents), infectious agents are likely to be one of the most important risk factors associated with abortions. A variety of sexually transmitted infectious agents has been reported to cause bovine abortion throughout the world (Tramuta et al., 2011). Hence, one of the main goals of the present communication is to review major pathogens associated with ST in cattle.

Infectious bovine rhinotracheitis (IBR). The infection with *Bovineherpesvirus*, leading to balanoposthitis in bulls and infectious pustular vulvovaginitis (IPV) in cows, has been recognized for several decades. It is a complex, baffling and changing disease, giving rise to much controversy, with prophylaxis proving difficult to devise and implement. The etiological agent, *Bovine herpesvirus 1* (BoHV-1) is a double-stranded DNA (dsDNA) virus and is a member of subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. The virus is not very resistant, and it is transmitted directly by respiratory, venereal or bucco-genital routes or indirectly via contaminated hands of a farmer or a semen collector. The infection can occur in both males and females without obvious clinical signs, or can be accompanied by non-specific inflammation, granulation tissue, vesicles and ulceration (which may be due to secondary infection with non-specific microorganisms) (Perez et al., 1985).

Bovine viral diarrhea (BVD). *Bovine viral diarrhea virus* (BVDV) is a *pestivirus* from the family *Flaviviridae* (Becher and Thiel, 2011), capable of causing serious clinical disease in cattle. The virus is divided into two genotypes (BVDV-1 and BVDV-2) on the basis of antigenic and genetic differences (Vilcek et al., 2005). Infection with BVDV is known to have a significant financial impact (Houe, 1999), stemming primarily from the reproductive and immunosuppressive effects of acute infection. The most common economic losses resulting from BVDV infection are associated with failure in fertilization, abortion, congenital malformation, stillbirth or birth of persistently infected (PI) progeny (Grahn et al., 1984; Rufenacht et al., 2001).

Chlamydial infections. Recognised for some time as the cause of epidemic abortion in cows in the USA, *Chlamydia psittaci* in Europe is associated with

sporadic abortion (at 3 to 7 months) due to necrotic placentitis and a direct effect on the fetus (producing liver lesions). Infertility associated with vaginitis and endometritis was also described.

Chlamydiae are obligate intracellular, gram-negative bacteria with atypical developmental life cycle. During the last ten years, four *Chlamydia* species have been isolated from humans and from different domestic and wild animals. Infection with chlamydial bacteria, specifically *C. pecorum*, *C. abortus*, and *C. psittaci*, are common in cattle. An infection with chlamydia may cause sub- or infertility. Infected bulls may suffer from vesiculitis, or may not be affected at all (Storz et al., 1968).

Mycoplasmoses. Different species of Mycoplasmas can affect bovines causing several diseases. Mycoplasmas can cause clinical, subclinical or chronic intramammary infection affecting cattle of all ages and at any stage of lactation (Tamiozzo et al., 2014). The numerous mycoplasmas involved in diseases of cattle create a complex situation with regard to lesions and the causal agent. Species commonly associated with genital tract infection in Europe are *Mycoplasma bovis genitalium*, *Mycoplasma bovis*, *Acholeplasma laidlawii* and *Ureaplasma spp.* Organisms of the genera *Mycoplasma*, *Acholeplasma* and *Ureaplasma* have been isolated from the distal part of both male and female bovine genital tract. *Mycoplasma bovis*, *M. bovis genitalium* and *U. diversum* appear to be the most important pathogens of the genital tract, involved in diseases such as decreased sperm motility (*M. bovis genitalium*), seminal vesiculitis, epididymitis (*M. bovis* and *M. bovis genitalium*), endometritis (*M. bovis*), granular vulvovaginitis, infertility and abortion (*U. diversum*) (Fish et al., 1985).

Solving the problem of animal pathology is the key to successful management of industrial livestock in Ukraine (Stegniy et. al., 2015). Most common infectious diseases transmitted via bull semen in Ukraine include infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVDV), chlamydioses, and mycoplasmoses. Hence, it is important to focus on the control of genetic resources in breeding work in order to effectively break the epizootic chain of the diseases.

MATERIALS AND METHODS

Monitoring studies for ST infections were conducted from 2013 to 2016. During this time, four hundred fifty (n=450) bull semen samples from 10 farms across Ukraine have been tested. PCR diagnostics was performed by the standard method developed in National Scientific Center "Institute for Experimental and Clinical Veterinary Medicine" (NSC «IECVM») (Stegniy et. al., 2015).

Isolation of total DNA was performed using a commercial kit for extraction of nucleic acids “DNA Sorb-B”, produced by “Central Research Institute of Epidemiology” (Russian Federation). Reverse transcription was performed using a set of reagents RT-Core production company IsoGene (Russian Federation).

The amplification reaction was performed using commercial set «PCR-Core» manufactured by IsoGene systems and primers (Table 1).

Table 1. Primers positive control samples and the size of amplicon generated for IBR, BVDV, and *Chlamydia*

	Primers	PCT	Product size (bp)
IBR	BOHVF_R	SL	202
BVDV	BVDVF_R	Oregon	267
<i>Chlamydia spp.</i>	CHLAMF_R	V.Olexandrivka\11	237
1. PCT - positive control template 2.SL - DNA formalin inactivated strain of IBR 3.V.Olexandrivka\11 - DNA boiling inactivated <i>Chl. psittaci</i> 4.Oregon - formalin inactivated strain of cattle BVDV.			

Identification of the BoHV-1, BVDV, *Chlamydia* and *Mycoplasma spp.* genetic material was performed according to standard amplification protocols.

RESULTS AND DISCUSSIONS

The analysis of research samples of cryopreserved semen from the bullsires for PCR revealed that genetic material of *Mycoplasma spp* was contained in 19 samples (Fig. 1) (6%), which came from farms in Kharkiv region, genetic material of BoHV-1 pathogen was detected in 2 (3.5%) positive samples from Poltava region, and 2 samples (4.5%) containing genetic material of the pathogen *Chlamydia spp* were obtained from Cherkasy region.

Table 2. Results of the study of bull semen in PCR 2013 – 2016 (n = 450)

Region	Re-search samples	The positive samples							
		BoHV-1		BVDV		<i>Chlamydia</i> spp.		<i>Mycoplasma</i> spp.	
		s	%	s	%	s	%	s	%
Kharkiv region	332	-	-	-	-	-	-	19	6
Poltava region	58	2	3.5	-	-	-	-	-	-
Zhytomyr region	13	-	-	-	-	-	-	-	-
Cherkasy region	44	-	-	-	-	2	4.5	-	-
Dnipropetrovsk region	3	-	-	-	-	-	-	-	-
s - sample									

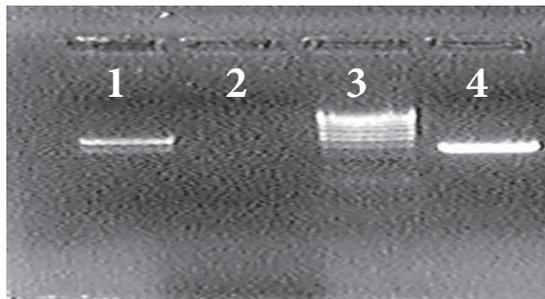


Figure 1. Results for of bull semen PCR detection on gel, positive sample of genetic material of *Mycoplasma* spp. Track number 1 – positive sample, lane number 3 – 100 base pair (bp) DNA ladder plus, lane number 4 – positive control.

We compared current research results of our study regarding frozen bull semen monitoring (period 2013-2016) with the results for period of 2010-2013. Significant reduction of contamination of semen samples with viruses, chlamydia and mycoplasmosis has been established (Fig. 2).

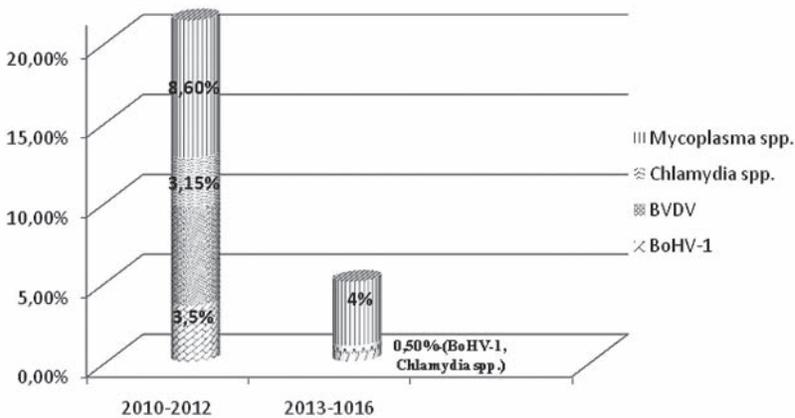


Figure 2. Comparison of PCR screening studies for the period 2010-2012 with the research conducted in the period 2013-2016.

CONCLUSIONS

According to the results obtained in this study, 19 samples (6%) from Kharkiv region contained genetic material of *Mycoplasma spp.* in frozen bulls semen samples, 2 samples from Poltava region (3.5%) were positive for BoHV-1, and 2 samples from Cherkasy region (4.5%) contained genetic material of *Chlamydia spp.*

Based on the monitoring results we can conclude that regular PCR screening of bulls' semen for the most common genital infections is positively affected by the dynamics of the testing rate. At present, there are no uniform requirements for contamination monitoring of sperm for the presence of viruses and Chlamydia. There are national requirements for the evaluation of semen contamination operating in Canada and the United States. Further work is therefore important to improve the system for control of contamination of cattle genetic resources.

REFERENCE

1. Becher, P., Thiel, H.J. Pestivirus (Flaviviridae). In: Tidona, C.A., Darai, G. (Eds.), Springer Index of Viruses, Second Ed. Springer Verlag, Heidelberg, Germany, 2011, pp. 483-488.
2. Eaglesome M.D., Garcia M.M.: Disease risks to animal health from artificial insemination with bovine semen, *Rev. sci. tech. Off. int. Epiz.*, 16, 1, 215-225, 1997.

3. Grahn T.C., Fahning M.L.: Zemjanis R. Nature of early reproductive failure caused by bovine viral diarrhoea virus. *J. Am. Vet. Med. Assoc.*, 185, 4, 429–432, 1984.
4. Houe, H.: Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Veterinary Microbiology* 64, 2/3, 89–107, 1999.
5. Fish N.A., Rosendal S, Miller R.B.: The Distribution of Mycoplasmas and Ureaplasmas in the Genital Tract of Normal Artificial Insemination Bulls, *Can Vet J*, 26, 1, 13-15, 1985.
6. Tamiozzo P.J., Estanguet A.A., Maito J., Tirante L., Pol M., Giraudo J.A.: Detection of Mycoplasma canadense and Mycoplasma californicum in dairy cattle from Argentina, *Rev Argent Microbiol*, 46, 2, 119-121, 2014.
7. Parez M.: The most important genital diseases of cattle (control, treatment and the hygiene of semen collection), *Rev. sci. tech. Off. int. Epiz.*, 4, 1, 69-87, 1985.
8. Rufenacht J., Schaller P., Audige L., Knutti B., Kupfer U., Peterhans E.: The effect of infection with bovine viral diarrhoea virus on the fertility of Swiss dairy cattle. *Theriogenology*, 56, 2, 199–210, 2001.
9. Stegnyy B., Gerilovich A., Bolotin V., Isakov M., Bashchenko M.: Detection of virus and bacterial contaminants of semen of pedigree bulls with the use of molecular – genetic methods, *News of agrarian science*, 2, 27-31, 2015.
10. Storz J., Carroll E.J., Ball L., Faulkner L.C.: Isolation of a psittacosis agent (Chlamydia) from semen and epididymitis of bulls with seminal vesiculitis syndrome. *Am J Vet Res*, 29, 3, 549-555, 1968.
11. Tramuta C., Lacerenza D., Zoppi S., Goria M., Dondo A., Ferroglio E., Nebbia P., Rosati S.: Development of a set of multiplex standard polymerase chain reaction assays for the identification of infectious agents from aborted bovine clinical samples, *Journal of Veterinary Diagnostic Investigation*, 23, 4, 657–664, 2011.
12. Vilcek, S., Durkovic, B., Kolesarova, M., Paton, D.J.: Genetic diversity of BVDV: Consequences for classification and molecular epidemiology. *Preventive Veterinary Medicine*, 72, 31–35, 2005.
13. Wentink G.H., Frankena K., Bosch J.C., Vandehoek J.E.D., Th van den Berg: Prevention of disease transmission by semen in cattle, *Livestock Production Science* 62, 3, 207–220, 2000.

Primljeno: 20.11.2016.

Odobreno: 25.12.2016.