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BOVINE RESPIRATORY DISEASE COMPLEX (BRDC): VIRAL AND BACTERIAL PATHOGENS IN SERBIA

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Abstract: Pathogens causing BRDC in Serbia were investigated. Two herds of beef cattle with bovine respiratory disease were included, with twenty diseased calves (10 from each farm) were chosen for isolation of bacteria on artificial culture media and determination by aerobic cultivation. The most common bacterial pathogen was isolated was *Pasteurella multocida.* Diffusion method of sensitivity to antibiotics (antibiogram), revealed that Enrofloxacin and Floron were most efficient antibiotics against *Pasteurella multocida* isolates (100 % isolates sensitive on both antibiotics). From the all examined samples (n=20) using the method of Real Time PCR (RT-PCR and PCR) we determined the genome sequences of bovine respiratory syncytial virus (BRSV), but in none of the samples genome of bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BoHV-1).

Key words: bronchopneumonia, viruses, bacteria, Real Time PCR, isolation, sensitivity on antibiotics.

Introduction

Bovine respiratory disease (BRDC) is a major disease problem for the cattle industry, and most costly disease with big economic losses: decreased production, higher levels of mortality and morbidity, increased labour costs and reduced carcass value *(Irsik et al., 2006)*. BRDC is multifactorial process, infectious agents including viruses, bacteria and mycoplasma *(Pardon et al., 2011; Duff et al., 2007; Ellis, 2001)*. Predisposing co-factors in the development of disease are: stress and environmental factors (weaning, temperature, stocking density, dust, humidity and shipping) and nutritional change *(Taylor et al. 2010; Snowder et al., 2006)*. Viral pathogens that causing primarily respiratory lessions are Bovine herpesvirus 1 (BoHV-1), Infectious bovine rhinotracheitis virus (IBRV), Bovine respiratory

syncitial virus (BRSV) and bovine parainfluenza Virus type 3 (BPI3V) *(Pardon et al., 2011; Lazić et al., 2009; Ellis, 2001)*. Unique among the bovine respiratory viral agents is BVDV, because intrauterine infection can lead in persistently infected (PI) cattle, chronically ill or dying in feedlots *(Loneragan et al., 2005; Kurćubić et al., 2011)*. Infection with BVDV lead to immunosuppression which causing progression of BRDC because facilitate invasion with opportunistic secondary pathogens such as *Mannheimia haemolytica* (16 serotypes), *Pasteurella multocida, Haemophilus somni* and a number of *mycoplasma* species such as *M. bovis* and *M. dispar (Pardon et al., 2011; Fulton, 2009; Hodgson et al., 2005; Ellis, 2001)*.

Molecular methods for identifying and sequencing the genomes of animal viruses are constantly updated, like developing of multiplex reverse transcription quantitative polymerase chain reaction (mRT-qPCR) assay (sensitive and specific technique capable of detection of three major viral respiratory pathogens of cattle by *Thonur et al., 2012*). Reagents for PCR assays are traditionally considered expensive, the ability to perform these assays within a short time frame to detect multiple pathogens can generate valuable information in differential diagnosis. Additional cost benefits on farm will result from more rapid diagnosis and the ability to target treatment, use appropriate vaccines or implement improved management procedures quickly. Molecular tests allow the assessment of development trends of microorganisms, a retrospective analysis of their geographical distribution and development of a database.

The aim of this study was to obtain basic knowledge of pathogens that cause BRDC in Serbia.

Materials and methods

Samples

Beef cattle, 5 months old, both sexes, Simmental race. Experimental animals are the property of "Kotlenik promet" d.o.o. Lađevci, from two farms, located near Čačak. From anamnesis and clinical examination on the day of sampling, we diagnosed the severe symptoms which justify suspicion of BRDC. Those symptoms were include: lose weight following a loss of appetite, visibly rapid breathing, lung auscultation tightened breathing, cough progresses to sound relatively dry, ocular and nasal discharge visible as either serous or yellow and viscous, depression and a progressive fever - rectal temperature above 40.1 ^{0}C.

Samples of discharge from the nasal mucosa were taken using sterile swabs and test tubes for the isolation of the etiological agents of viral and bacterial origin, from clinically dieseased and health animals in same herd (facilities). All samples were shipped in a hand portable refrigerator within one hour after sampling to the acredited microbiological laboratory of Veterinary Specialist Institute "Kraljevo".

Isolation of bacterial organisms on artificial culture media was determined by aerobic cultivation, with subsequent biochemical identification and determination of the isolated strains (*Dujin et al*., *1984*).

Determination of bacteria presence

Sensitivity testing of isolated bacterial strains to antibiotics and sulfonamides (antibiogram) was performed using the disk diffusion method, according to Kirby-Bauer procedure (1966).

Real Time PCR (RT-PCR) method

a) Determining the presence of genome of IBRV

The genome of the virus IBRV was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany). The real-time PCR reaction was performed using the Maxima™ Probe qPCR Master Mix (Fermentas, Lithuania), the Real Time PCR machine MX3000P Strategene, according to the protocol described in the OIE Manual, Chapter 2.4.13. *(OIE, 2008)*.

b) Determination of the BVDV and BRSV presence

BVDV genome was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). Real time RT-qPCR reaction was performed using the Superscript III Platinum® One-Step Quantitative RT-PCR System (Invitrogen, USA), the Real Time PCR machine MX3000P Strategene. BVDV genomic RNA presence was done according to the protocol described by *Baxi et al. (2006)*, and detection and quantitation of BRSV was done with the protocol described by *Boxus et al. (2005)*.

Results and Discussion

Results of the microbiological tests and susceptibility testing are presented in the tables below (1-2).

From 10 swabs taken form nasal mucosa of beef cattle on farm 1, five animals (50 %) were positive on bacteriological examination (aerobically). Presence of the agents in positive beef cattle is as follows: *Pasteurella multocida* (5/50 %); *Aeromonas viridans* (4/40 %); *Corynebacterium bovis* (3/30 %); *Micrococcus luteus* (2/20 %); *Mannheimia haemolytica* (1/10 %). On farm 2, six animals (60 %) were positive on bacteriological examination (aerobically). Presence of the agents in positive beef cattle is as follows: *Pasteurella multocida* (6/60 %); *Corynebacterium bovis* (4/40 %); *Aeromonas viridans* (3/30 %); *Mannheimia haemolytica* (2/20 %); *Micrococcus luteus* (1/10 %).

Determination of the infectious bovine rhinotracheitis virus (IBRV) and BVDV genome presence revealed that the presence is not established, in all 20 examined samples (animals), from both farms. Genome of the BRSV is determined in all 20 examined samples, from both farms, and confirmed the findings of *Brodersen (2010)* that the BRSV is a major cause of respiratory disease and a major

contributor to the BRDC. Our result is in accordance to the observed clinical signs in diseased beef cattle.

Predominant isolated bacteria from nasal swabs were *Pasteurella multocida*, and the results of the isolates sensitivity to antibiotics and sulfonamides (antibiogram) is showen in table 2.From data presented in table 2, we can conclude that the most efficient antibiotics against *Pasteurella multocida* isolates were Enrofloxacin and Floron (11/100 % isolates sensitive on both antibiotics).

Table 1. Pathogens detected in the samples of nasal swab from beef cattle (FARM 1 and 2)

	Values																			
	No. of samples (Farm 1)										No. of samples (Farm 2)									
Parameter	1013	8979	7102	355	1152	7137	444	305	7119	58	8380	9478	603	6721	9171	9923	8994	1700	7756	9829
Bacteriological examination (aerobically)	+	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	+
Mannheimia haemolytica	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+
Pasteurella multocida	+	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	+
Aeromonas viridans	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+	-	-	-
Micrococcus luteus	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
Corynebacterium bovis	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	-	-	-
IBR/IPV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BVDV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRSV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. *Pasteurella multocida* isolates sensitivity to antibiotics and sulfonamides (antibiogram)

Antibiotics	Farm 1					Farm 2					
	IS 1*	IS 2	IS 3	IS 4	IS 5	IS 6	IS 7	IS 8	IS 9	IS 10	IS 11
Amoxicillin	S	R	R	S	S	S	S	S	S	S	S
Ampicillin	S	R	R	S	S	S	S	S	S	S	S
Enrofloxacin	S	S	S	S	S	S	S	S	S	S	S
Gentamycin	I	R	S	I	S	I	R	I	I	I	I
Neomycin	S	S	S	S	S	-	-	-	-	-	-
Penicillin	I	R	R	S	S	-	-	-	-	-	-
Tetracycline	I	R	R	S	I	I	I	S	S	S	S
Trimetoprim+sulphometoxasol	I	R	R	S	S	I	R	I	S	S	I
Floron	S	S	S	S	S	S	S	S	S	S	S
Tylosin	-	-	-	-	-	I	I	I	I	I	I

Legend:IS 1* - no. of isolates; S - Sensitive; I - Intermediate sensitivity; R - Resitant.

Conclusion

The most common bacterial findings were *P. multocida*, *Aeromonas viridans* and *Corynebacterium bovis*, suggesting on their higher importance in BRDC in Serbian beef cattle with regard to *Mannheimia haemolytica*, predominantly determined worldwide. How these three pathogens interact together and with viruses remains to be clarified. According to Real-time RT-PCR and PCR findings BRSV is common virus present in Serbian beef cattle herds suffering from BRDC (BoHV-1 and BVDV genome were not identified in our study). Enrofloxacin and Floron were found to be the most efficient antibiotics against *P. multocida* isolates were 100 % of examined isolates sensitive on both antibiotics).

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Kompleks respiratornog oboljenja goveda: virusni i bakterijski uzročnici u Srbiji

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Rezime

Ispitivani su patogeni koji izazivaju BRDC u Srbiji. Iz dva stada tovne junadi smo odabrali dvadeset junadi obolelih od BRDC (po 10 sa svake farme) od kojih smo uzeli uzorke za izolaciju bakterija na veštačkim podlogama i određivanje aerobnom kultivacijom. Najčešće izolovana bakterija je bila *Pasteurella multocida*. Testiranje osetljivosti izolovanih bakterijskih sojeva na antibiotike i sulfonamide je realizovano difuzionom metodom (antibiogram), a najefikasniji antibiotici protiv izolata *Pasteurella multocida* su Enrofloxacin i Floron (100% izolata osetljivih na oba antibiotika). Od svih ispitivanih uzoraka (n = 20) sa obe farme metodom Real Time PCR (RT-PCR and PCR), ustanovili smo sekvence genoma bovinog respiratornog sincicijelnog virusa (BRSV), ali ni u jednom od uzoraka genom virusa bovine virusne dijareje (BVDV) i herpesvirusa-1 goveda (BoHV-1).

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