REVIEW ARTICLE

Modern Approach to the Enigma of Bovine Respiratory Disease Complex: A Review

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ABSTRACT

Research on bovine respiratory disease complex (BRDC) has strongly contributed to a better understanding of disease at both global and local levels. New agents have been identified and their nature has been better known since then, as well as all the factors that predispose the development of BRDC. Serious work in a number of researches has led to results that have proven significant synergistic interactions between viruses and bacteria that lead to the BRDC and complicate the prevention and treatment of the diseased cattle. Nowadays, it is necessary to apply the latest molecular and other techniques in order to examine in detail all of the specific causes, describe them accurately and provide effective prevention. This can primarily be accomplished by producing different types of vaccines, which are to be improved constantly. Serious progress has been made in researches on immunogenic properties and antimicrobial activity of various natural and synthetic substances in order to improve the production of more effective vaccines for control, as well as the development of a new generation of antibiotics to treat the disease. BRDC prevention programs have been successful, in which great importance was given to proper vaccinations and herd management practices. BRDC is still the most important disease of cattle from an economic point of view, since it causes great losses and expenses.

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INTRODUCTION

The bovine respiratory disease complex (BRDC) is a multi-factorial illness with many variables that in synergy cause a disease which usually leads to severe and fatal outbreaks of bacterial pneumonia. These facts make it difficult to control the BRDC, especially in the categories of beef calves before and after weaning (Lazić *et al*., 2004). Numerous studies around the world have demonstrated a significant role of predisposing factors in the development of BRDC (Taylor *et al*., 2010), included environmental (ambient temperature, humidity, ventilation, harmful gases and dust) and host factors (age, sex, breed, genetics, immune status and concurrent disease). Stressful management practices (weaning, shipping, changes in diet, high density of animals, handling and surgeries) carry a high risk of BRDC appearance in cattle herds. The most commonly noticed clinical symptoms are: high fever (about 40-41.5ºC),

decreased appetite, depression, nasal and ocular discharge, coughing and different degree of dyspnea (Urban-Chmiel and Grooms, 2012). Among a large group of infectious agents which participated in the pathogenesis of BRDC, the most common viruses involved in BRDC include Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR), Bovine Respiratory Syncytial Virus (BRSV), and Para influenza Type-3 Virus (PI-3V) which can cause severe damage to the respiratory tract of cattle, creating opportunities for settlement of bacteria in the lungs (Pardon *et al*., 2011). Adherence of bacteria (*M. haemolytica*) to bronchial epithelial cells causes the most acute form with progressive inflammation characterized by fibrinous bronchopneumonia. Intrauterine infection of cattle with non-cytopathogenic (ncp) BVDV in the first trimester of gestation is unique, and can play a role in the emerging of persistently infected (PI) cattle, chronically ill or dying in feedlots (Peterhans *et al*., 2010). Latest research in this field (Raaperi *et al*., 2012) reveals opinion

that BHV-1 was not associated with acute respiratory disease in adult dairy cattle, but it caused significant changes in reproductive performances. Many authors believe that the BRSV has a major role in the onset of BRDC in adult dairy cattle. The importance of bovine adenovirus, coronavirus, calicivirus, parvovirus, herpesvirus type 4, reovirus and rhinovirus is still considered episodic (Lazić *et al*., 2009).

The most common bacteria found in lungs of calves with BRDC include *Mannheimia haemolytica* and *Pasteurella multocida*. *Haemophilus somnus* may also be involved in cases of pneumonia and can cause severe damage to heart muscles. *Mycoplasma bovis* have the potential to further complicate the BRDC pathogenesis (Griffin *et al*., 2010). Many researchers have examined the impact of various microbial agents and their interaction in the development of the clinical outbreaks of BRDC, vaccination trials (Wildman *et al*., 2008), serological (Loneragan *et al*., 2005), and molecular surveys (Thonur *et al*., 2012; Fratrić *et al*., 2012). The appearance of clinically affected animals does not typically differ significantly regardless of the pathogen involved. Viral agents may occasionally produce a clinical syndrome consistent with BRD without bacterial co-infection (Decaro *et al*., 2008). BRDC is one of the most costly diseases due to a decline in productivity, higher levels of mortality and morbidity (Loneragan *et al*., 2001; Zahur *et al*., 2007), increased labor costs and reduced carcass value (Schneider *et al*., 2009; Garcia *et al*., 2010). BRDC is the most common cause of mortality in dairy cattle, with highest morbidity and higher mortality, which are noticed in case of mixed viral and bacterial infections (Duff and Galyean, 2007). Diagnosis of BRDC can be achieved through routine clinical examination and observation, quantified in the form of a clinical score. The second diagnostic method is to use a series of laboratory tests available to identify microbial agents involved.

Bovine Herpesvirus 1 (BHV-1): BHV-1 has an outer layer (peplos) and belongs to the genus of *Varicellovirus*, subfamily of *Alphaherpesvirinae,* and family of *Herpesviridae* (Šamanc *et al*., 2009). Strains of BHV-1 have been classified into five subtypes, according to the results of testing their genome: 1, 2a, 2b, 3a and 3b (Đurišić *et al*., 2003). Subtype 1 of BHV-1 was isolated in cases of IBR, subtype 2 in genital disease accompanied by the appearance of rashes and inflammation of the mucous membrane of the vulva and vagina of cows (infective pustular vulvovaginitis - IPV), and subtype 3 in cases of neuralgic disease (bovine encephalitis herpesvirus - BEHV). Balanoposthitis in bulls, abortions and fatal multi-systemic infectuous clinical forms in cattle are described as a result of BHV-1 infection (Nišavić *et al*., 2010; Takiuchi, 2005). Prevalence of BHV-1 is even more than 50% in Serbia (Lazić *et al*., 1995). Lazić *et al*. (2001) investigated the humoral immunological response in 26 cows vaccinated with anti BHV-1 vaccine ("Borinak", Mevak, Nitra, Slovak Republic). Prior to vaccination, the values of Ab titers ranged from 1:4 to 1:64. At the time of parturition they ranged from 1:64 to 1:1024 in blood sera, and from 1:256 to 1:2048 in colostral sera. Specific Ab for BHV-1 was determined in 2 calves from these cows before feeding with colostrums. After feeding with

colostrum the highest values for Ab titers were found in 2 days old calves, while the lowest values were noticed in 90 days old calves. Lazić *et al*. (2003) conducted a preliminary serological testing of 10 cattle herds and found that the infection was caused by BHV-1 was present in 8 herds, and the percentage of seropositive animals tested ranged from 37 to 98%. Lazić *et al*. (2004) report that an infection caused by a virus PI-3 was present in all 10 herds tested, and infections caused by IBRV and BVDV in 8 herds. Lazić *et al*. (2008) examined the influence of number of animals per household on epizootiology of BHV-1 infection. Households were classified according to the number of animals on one farm: 6 to 20, 21 to 50 and more than 50 animals in one herd. Prevalence of BHV-1 infection was estimated and led to a conclusion that the more animals raised in a household, the higher the number of cattle infected with BHV-1. Turnover of infected breeding animals from large herds spread BHV-1 infection to small herds of cattle. Šamanc *et al*. (2009) conducted comparative testing of 92 samples of blood serum of unvaccinated calves and heifers for the presence of Ab against BRSV, PI-3 and BHV-1 by indirect enzyme immunoassay (iELISA), revealed the presence of Ab against BHV-1 in 19 (20.65%) of the samples.

Diagnosis of BHV-1 infection was usually done by isolating the virus, detection of viral antigens by using the ELISA or immunofluorescence technique. Polymerase chain reaction (PCR) is one of the nucleic acid hybridization techniques capable to detect DNA of three major viral respiratory pathogens of cattle (Thonur *et al*., 2012). Nišavić *et al*. (2010) isolated the BHV-1 from bovine nasal and ocular swabs, as well as from lungs, trachea and tonsils samples and identified it by virus-neutralization test (VNT). Further identification was done by PCR assay based on two pairs of primers selected from the sequence of gB glycoprotein gene which amplified a 478 bp fragment and thymidine kinase gene which amplified an 183bp fragment. Comparative analysis of DNA fragments of controlled laboratory strain TN 41 of BHV-1 and isolated strains obtained by PCR with primers for viral thymidine kinase gene coding region, confirmed that the isolated strain belongs to BHV-1.

Bovine Respiratory Syncytial Virus (BRSV): BRSV belongs to the genus of the *Pneumovirus*, subfamily of *Paramyxovirinae,* and the family of *Paramixoviridae* (Šamanc *et al*., 2009). Quinting *et al*. (2007) stated that the disease usually occurs in cattle younger than 18 months and calves at the age of 10 days to several months. Bugarski *et al*. (2011) conducted serological examination on dairy farms and cattle feedlots in Vojvodina on BRSV infection and revealed that they cause 60-70% of outbreaks of pneumonia in dairy and beef cattle, with different rates of mortality. Determination of the titer of Ab against BRSV in the blood sera was performed by VNT, using BRSV strain A5198 (ATCC USA). One of the dairy farms showed 100% of seropositive animals. From a total of 223 tested serum samples from feedlots, 60.09% contained Ab against BRSV, and seroprevalence depending on the phase of fattening. The results of testing 92 samples of blood serum of cattle in Serbia using iELISA confirmed 40 samples of Ab against the BRSV

and PI-3 virus (Šamanc *et al.*, 2009). Kurćubić *et al.* (2012) determined genome of the BRSV by Real-time RT-PCR in all 20 examined samples of discharge from the nasal mucosa of the diseased beef cattle from two experimental farms, and confirmed findings of Brodersen (2010) and Raaperi *et al.* (2012), stating that the BRSV is a major cause of respiratory diseases and a major contributor to the BRDC.

Bovine Viral Diarrhea Virus (BVDV): BVDV belongs to the family of *Flaviviridae* in the genus *Pestivirus* (Wegelt, 2011). Ridpath and Fulton (2009) revealed that diversity of BVDV reflected through the 12 BVDV1 subgenotypes (BVDV1a to BVDV11) and two BVDV2 subgenotypes (BVDV2a and BVDV2b), hamper the production of effective vaccines and could potentially complicate the reading of the diagnostic tests results. BVDV can be divided into the cytopathogenic (cp) and non-cytopathogenic (ncp) biotypes, based on the presence or absence of the visible cytopathic effect in infected cell cultures. PI virus carriers with a long-term life play the key role in BVDV epidemiology.

The presence of BVDV infection in Serbia has unofficially confirmed earlier (Kurćubić, 1993; Petrović, 2002; Petrović *et al.*, 2004; Petrović, 2006; Mahmood *et al.*, 2012). Kurćubić (1993) conducted study on dairy farms where the percentage of BVDV seropositive animals varied between 30.55 and 52.24%, depending on the age category and herd management. On the second farm with beef cattle (aged 6 to 7 months) 55.81% of seropositive sera was revealed. Petrović *et al.* (2004a; 2004b) detected the ncp BVDV isolates 0016 and 0017 and the cp isolate Beograd by indirect immunostaining methods. Official registration of BVDV infection of cattle in Serbia and Montenegro was received from the OIE reference laboratory (Veterinary Laboratories Agency, Weybridge, UK - letter of December 9, 2002). Direct sequencing of PCR products, alignment and phylogenetic analysis showed that the 0016 and Beograd isolates were of genetic subtype BVDV-1f (first report of a cp BVDV belonging to the 1f subgroup), whilst the 0017 isolate belonged to subtype 1b. Petrović (2006) examined 12083 blood samples of cattle older than 6 and the presence of specific Ab against BVDV was established in 4647 (38.46%) of the tested animals.

Ahmad *et al.* (2012) confirmed that both the serum and ear notch biopsy can be used for the detection of PI and genotyping of prevailing BVDV, but ear notch biopsies had less sensitivity (P<0.05) for the detection of acute transiently infected cattle than serum samples.

Basic approach in BVDV infections control is vaccination (Raue *et al.*, 2011; Luo *et al.*, 2012) and another approach is identification of PI cattle and their removal from the herd, without vaccination. Kurćubić *et al.* (2009) made fisrst attempt on identification of cattle PI with the BVDV among the dairy cattle that were seronegative in both examination of paired sera (first and 28. days), allow the ELISA testing to determine the BVDV antigens (Ag) in serum. BVDV Ag was not confirmed in either case. In the same manner, Kurćubić *et al.* (2010a) tried to identify the presence of PI in cattle of various ages from herds with a different production purpose (fattening/milk) and BVDV Ag was not

confirmed in either of the cases. The explanation for the lack of experimental proof of the presence of PI cattle can be found in the fact that the not tested sera of all animals from the farms on presence of BVDV Ag, whose age allowed this examination, as well-known fact that the prevalence of PI cattle was extremely low (0.75-2%). Kurćubić *et al.* (2010b) used VNT to examine blood sera collected from sheep of different age categories reared under different systems for the presence of BVDV and Border disease virus (BDV) infections in attempt to clarify the potential for transmission to cattle and *vice versa*. From animals breed on five mini farms that were not exist cohabitation between small and big ruminants in the same facilities and 5 sheep farms whose owners also raised cattle. Samples of blood were taken from 5 young sheep aged up to 12 months and 5 from the older sheep per farm. Serological examinations did not detect the presence of anti-BVDV specific Ab to BVDV-1 and BVDV-2 genotypes in any of the 100 tested blood serum samples, like anti-BDV Ab to BDV Moredun strain.

Parainfluenza Type-3 Virus (PI-3V): PI-3V is an immunosuppressive RNA virus belonging to the *Paramyxoviridae* family. PI-3V is usually transmitted in nasal discharge, favored by close contact, insufficient ventilation in facilities and stale air during shipping of cattle. The PI-3V was detected in acute and chronicle cases of pneumonia predominantly in young cattle during fall and winter. PI-3V often takes part in mixed infections with BHV-1 and BVDV, with severe clinical signs and fatal outcome. Fever, cough, mucous yellow nasal and eye discharge were noticed during the mild course of the disease. Milić *et al.* (2003) committed biochemical characterization of the surface glycoprotein Ag of purified PI-3V virions with molecular weights of 112 kD, 81-82 kD and 30-31 kD (HN and F Ag). After that, the activation of PI-3V followed with trypsin-versen representing a prerequisite for the application of the cell fusion test, hemolytic test and the test of inhibition of cell fusion with hyper immune sera against PI-3V can be used for fast detection and identification of fusion and hem agglutinin-neuraminidase PI-3V Ag. Lazić *et al.* (2009) indicate that in Serbian literature there are only few data on PI-3V as the causative agent of BRDC, especially in beef cattle. Serological surveys of PI-3V infection are strongly recommended. His research presented the BRDC outbreak accompanied by PI-3V infection in one bull calves fattening unit of industrial type. The geometric mean value of Ab titer against PI-3V was 14.58 at the moment of disease, while 3 and 6 weeks after outbreak it was 45.25 and 54.44, and at the end of fattening it was 22.11. Šamanc *et al.* (2009) state that the results of testing of 92 blood serum samples of cattle using iELISA confirmed the presence of Ab against PI-3V in 77 of samples (83.69%).

Bacterial agents: The most common bacteria found in the lungs of cattle with BRDC are *Mannheimia haemolytica* and *Pasteurella multocida*. Fulton (2009) states in his review that sixteen different serotypes of biotype A and T of *Mannheimia haemolytica* were classified, with twelve different serotypes based on capsular serotypes for the A serotype. High immunogenic potential for incorporation in

Fig. 1: Prominent fibrinous pleuritis and pleural effusion. The pulmonary interlobular septa are distended by yellow gellatinous edema and fibrin. (Courtesy: Nikola Vaskovic, Veterinary Specialized Institute, Kraljevo, Serbia).

P. multocida vaccine have leucotoxin produced by recombinant DNA technology and outer membrane protein PlpE. Today's researchers opinions based on the experimental evidence that the *P. multocida* is primary pathogen in the lungs of cattle have been confirmed in five-years research of Kurćubić *et al.* (2000). They examined a total of 1435 nasal swabs originating from diseased beef cattle with the symptoms that indicate BRDC. The most commonly isolated are *Pasteurella multocida* and *Pasteurella haemolytica* (62.78 and 40.20%), as well as *Corynebacterium pyogenes* (53.44%), *Staphylococcus albus* (24.87%) and *Streptococcus viridans* (14.07%). Hellenbrand *et al.* (2012) report that *Histophilus somni* is a Gram-negative pleomorphic coccobacillus that causes respiratory, reproductive, cardiac and neuronal diseases in cattle. Recently, it was discovered that bovine neutrophils and macrophages produce extracellular traps in response to *Mannheimia haemolytica*, which suggest that extracellular traps may play a role in the host response to *H. somni* infection in cattle. *Mycoplasma bovis* is a bacterial pathogen causing respiratory disease, otitis media, poly-arthritis, mastitis, and a variety of other diseases in dairy and beef cattle, with an important impact on the health, welfare, and productivity. Difficulties in implementation of *M. bovis* diagnostic and control procedures originate from very different manifestation of the disease, response to vaccination and medication, and lacks in knowledge about these diseases (Maunsell *et al.*, 2011).

Pathology: The gross lesions in the case of pneumonic Mannheimiosis are characterized by fibrinous bronchopneumonia, with prominent fibrinous pleuritis and pleural effusion. The interlobular septa is distended by yellow, gellatinous edema and fibrin (Fig. 1). Coagulation necrosis interlobular interstitial oedema and congestion are present as well. Microscopically first neutrophils fill the bronchial, bronchiolar and alveolar spaces, and then alveolar macrophages start to appear in the lungs (Fig. 2). Lung lessions caused by viruses in acute phases are those of bronchointestitial pneumonia in almost all the animal species (Fig. 3) (Marinković *et al.*, 2007). Microscopically, the lesions are in range of necrotizing bronchiolitis, necrosis of type I pneumocytes with hyperplasia of type II pneumocytes, and mild interstitial
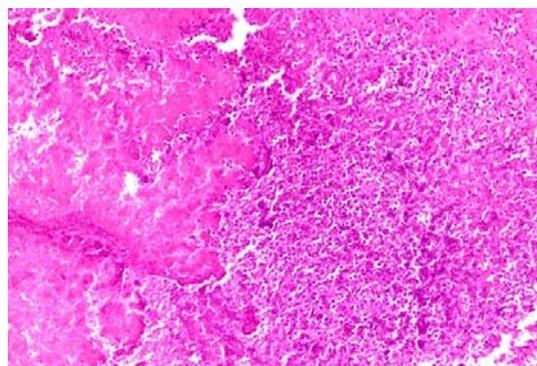

Fig. 2: Microscopically neutrophils fill the bronchial, bronchiolar and alveolar spaces, alveolar macrophages start to appear in the lungs, H&E, 200 ×. (Courtesy: Natalija Fratric and Sanja Aleksic-Kovacevic, University of Belgrade, Serbia).
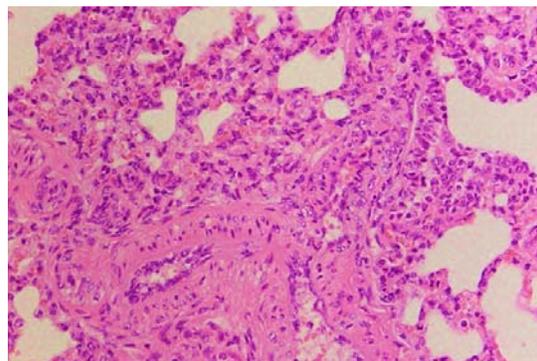

Fig. 3: The lesions are composed of necrosis of type I pneumocytes with hyperplasia of type II pneumocytes, and interstitial edema, H&E, 400 ×. (Courtesy: Natalija Fratric and Sanja Aleksic-Kovacevic, University of Belgrade, Serbia).
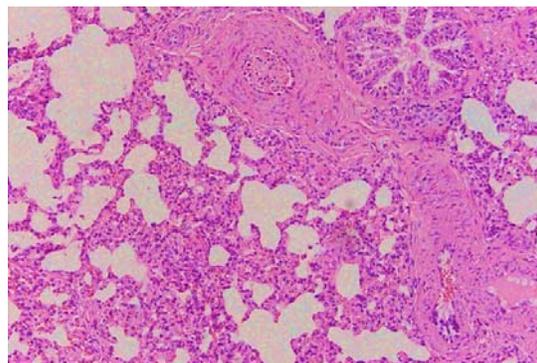

Fig. 4: The mycoplasmal also cause Bronchiolitis and prominent interstitial reaction. Numerous mononuclear inflammatory cells are present. H&E, 200 ×. (Courtesy: Natalija Fratric and Sanja Aleksic-Kovacevic, University of Belgrade, Serbia).

and alveolar edema. The mycoplasmal also cause bronchiolitis, bronchiolar and alveolar necrosis, and interstitial reaction (Fig. 4). A mixture of desquamated epithelial cells, macrophages, and mononuclear cells are usually present in the lumen of bronchioles and alveoli.

Vaccines and Vaccination Programmes: Significant achievements in vaccinology in the last 40 years have not yet enabled production of an ideal vaccine, which is expected to be multivalent, to create a protective immune response in neonatal calves regardless of the presence of

colostral antibodies, ensure long immune protection after the single vaccination, not to cause local and/or systemic reactions, and not to be applied by the syringe and injection needle (Rogan, 2010). Researchers have studied the impact of lincenced and experimental vaccines on morbidity, mortality, and other control measures in cattle (Larson and Step, 2012). Kurćubić et al. (2011a) evaluate the immunogenicity of two experimentally prepared inactivated vaccines (mono and polyvalent) containing BVDV reference and field strains. Three experimental groups were formed: 10 calves vaccinated twice (days 1 and 28) s.c. with 2 mL of inactivated polyvalent vaccine per animal (Group I); 10 calves vaccinated in same manner with inactivated monovalent vaccine (Group II) and 9 unvaccinated calves (Control, Group C). Blood sera were obtained from the immunized animals by standard procedure: on days 0, 14, 28, 42 and 56 post immunization, and tested by VNT. The immune response developed more rapidly and values of geometric mean titer (GMT) for BVDV neutralizing Ab were substantially higher in blood sera of calves from Group II. In the second part of the study (Kurćubić et al., 2011b), the immune response to same BVDV vaccine was evaluated by the iELISA method. The occurrence of a specific Ab against BVDV was first detected on day 42 post immunization in both experimental groups of beef calves and remained steady until day 56. The statistically very significant differences (at the 99 % level) observed between the test animals of groups I and II on days 42 and 56 after vaccination suggest considerably higher immunogenicity of the monovalent vaccine. Đurišić et al. (2003) report that large pharmaceutical companies developed a vaccine manufacturing technology in which the viral genome is partially shortened or part of the genome is cut and incorporated into the genome of other living systems such as cell cultures to produce precisely defined glycoprotein (marker vaccines) which allows the identification of vaccinated and infected animals. There are two vaccines for active immunization of healthy cattle and calves against respiratory disease currently available in Serbia, produced by the only domestic manufacturer. Vibak[®] is combined, polyvalent vaccine (ATCvet code QI02AF) containing live attenuated viruses (IBRV and PI-3V) and cell culture of bacteria *Diplococcus pneumoniae*, *Pasteurella multocida* and *Corynebacterium pyogenes*. The other is inactivated oil vaccine Respi-Ol[®] (ATCvet QI02AD06), containing the immunogenic strains of PI-3V and IBRV. The only available registered import vaccine is Bovilis[®] IBR marker inac (Intervet International B.V., ATCvet QI02AA03), which contains inactivated BHV-1 strain GK/D.

Medication: The therapeutic approach for BRDC includes antimicrobial treatment due to the frequent participation of bacteria. The data concerning the use of ancillary medications (nonsteroidal anti-inflammatory drugs, immunomodulators, bronchodilators) are often based on experimental models of BRDC (Francoz et al., 2012). Spreading of infective bacterial agents which infect the cattle with BRDC can be prevented by the use of antimicrobial agents when the number of "high risk" calves reach the "tolerance level". Drugs for metaphylaxis (mass medication) were selected on the basis of

susceptibility testing, mainly with prolonged action, for the treatment of animals who were suspected of BRDC which have been in contact with the infected cattle and gave good results in reducing morbidity (Edwards, 2010; Urban-Chmiel and Grooms, 2012). The new macrolide antibiotics, third generation cephalosporins, fluoro-quinolones and fenicols showed high clinical success (70-90%) in the treatment of cattle suffering of BRDC caused by *Pasteurella's*, 1-2 days after treatment. Tulatromycin, florfenicol, ceftiofur are much more effective against most *Pasteurella* strains that are resistant to other antimicrobial drugs. Its liposolubility allowed a quick and complete absorption in the lung parenchyma, held there for seven days at concentrations higher than the minimal inhibitory concentration (MIC) for most bacteria and mycoplasma. Jezdimirović et al. (2011) examined the clinical efficacy of tulathromycin (TU) and florfenicol (FL) in the treatment of bronchopneumonia (BP) caused by *Pasteurella multocida* isolated from diseased six months old Holstein calves. TU proved to be a drug of first choice in the treatment of BP caused by *P. multocida*. Recent findings of Kurćubić et al. (2012) revealed that the most common bacteria in fattening cattle suffering from BRDC were *P. multocida*, *Aeromonas viridans* and *Corynebacterium bovis*, suggesting their higher importance in BRDC in Serbian beef cattle in regard to *Mannheimia haemolytica*, predominantly determined worldwide. Susceptibility testing revealed that the most efficient antibiotics against *P. multocida* isolates were enrofloxacin and florfenicol (100% of examined isolates sensitive on both antibiotics). Portis et al. (2012) tested bovine isolates of *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, collected over the last ten years to examine the *in vitro* sensitivity of the commonly applied *ad us vet* antibiotics that are available in the market. Ceftiofur remained very active against all isolates. Penicillin retained good activity against *P. multocida* and *H. somni* isolates. Enrofloxacin MIC_{50} values for the 3 pathogens also did not change over time, unlike the MIC_{90} values, which increased by at least 4-doubling dilutions. Ninety percent or more of *M. haemolytica* and *H. somni* isolates were susceptible to florfenicol, while susceptibility among *P. multocida* was 79% or higher. All 3 organisms showed declines in tilmicosin and tulathromycin MIC_{50} and MIC_{90} values.

Guidelines for Future Research: One of the most important tasks for the researchers to determine now is the trend of resistance of isolated bacteria to antibiotics used in clinical veterinary practice. If they detect multiresistant pathogens listed diseases, with sampling performed from healthy individuals who were not under antibiotic treatment, with the aim to isolate non-pathogenic strains sensitive to all antibiotics, which could be embedded in a new experimental vaccines. It is essential that a detailed analysis of selected characteristic sequences determine the position of the bacteria and viruses that lead to BRDC in the phylogenetic tree in relation to the same strains in the environment and then form a database. Phylogenetic analysis based on the comparison of the nucleotide/amino acid sequences of specific genetic determinants are an extremely powerful tool in modern approach in epizootiology, in order to determine the genetic diversity,

assess the evolutionary flow, a retrospective analysis of the geographical distribution of different antigenic types and subtypes of analyzed microbial agents was carried out. Vaccines and diagnostic manufacturers are interested in the results of these studies. Improvement of the existing or development of new vaccines can incorporate native and current determined viral and bacterial specific antigens, which are described and standardized by molecular methods.

Conclusion: Contemporary approach for BRDC researchers and veterinary clinicians in Serbia is to enable faster and more accurate (field) diagnosis in order to get a better prevention of the disease and achieve a significant reduction in economic losses. The ultimate goal is to improve the beef meat production intended for human consumption. It is necessary to study in detail a large number of predisposing environmental factors and applications of obtained results in adjustment of the herd management, taking into account the principles of animal welfare. It is imperative to properly design vaccination programs and reduce the usage of antibiotics, especially the drugs used routinely in human medicine.

REFERENCES

Ahmad A, M Rabbani, MZ Shabbir, K Muhammad, T Yaqub, MK Saleemi, RK Khalid, MAB Shabbir and SY Alvi, 2012. Comparative suitability of ear notch biopsy and serum pairs for detecting nature of bovine viral diarrhoea virus infection in dairy herds. Pak Vet J, 32: 451-455.
Brodersen BW, 2010. Bovine Respiratory Syncytial Virus. Vet Clin N Am-Food A, 26: 323-333.
Bugarski D, T Petrović, D Milanov and S Lazić, 2011. Seroprevalence of Bovine Respiratory Syncytial Virus (BRSV) in Vojvodina. Arh Vet Med, 4: 23-29.
Decaro N, M Campolo, C Desario, F Cirone, M D'Abramo, E Lorusso, G Greco, V Mari, ML Colaianni, G Elia, V Martella and C Buonavoglia, 2008. Respiratory disease associated with bovine coronavirus infection in cattle herds in Southern Italy. J Vet Diagn Invest, 20: 28-32.
Duff GS and ML Galyean, 2007. Recent advances in management of highly stressed newly received feedlot cattle. J Anim Sci, 85: 823-840.
Đurišić S, S Lazić, T Petrović, D Lupulović and M Velhner, 2003. Latest Knowledge on major biological characteristics of bovine Herpes Virus-1. Vet Glas, 57: 439-448.
Edwards TA, 2010. Control methods for bovine respiratory disease for feedlot cattle. Vet Clin N Am-Food A, 26: 273-284.
Francoz D, S Buczinski and M Apley, 2012. Evidence related to the use of ancillary drugs in bovine respiratory disease (anti-inflammatory and others): are they justified or not? Vet Clin N Am-Food A, 28: 23-38.
Fratrić N, D Gvozdić, D Vuković, O Savić, M Buač and V Ilić, 2012. Evidence that calf bronchopneumonia may be accompanied by

increased sialylation of circulating immune complexes' IgG. Vet Immunol Immunopathol, 150: 161-168.
Fulton RW, 2009. Bovine Respiratory Disease Research (1983-2009). Anim Health Res Rev, 10: 131-139.
Garcia MD, RM Thallman, TL Wheeler, SD Shackelford and E Casas, 2010. Effect of bovine respiratory disease and overall pathogenic disease incidence on carcass traits. J Anim Sci, 88: 491-496.
Griffin D, MM Chengappa, J Kuszak and DS McVey, 2010. Bacterial pathogens of the bovine respiratory disease complex. Vet Clin N Am-Food A, 26: 381-394.
Hellenbrand KM, KM Forsythe, JJ Rivera-Rivas, CJ Czuprynski and NA Aulik, 2012. *Histophilus somni* causes extracellular trap formation by bovine neutrophils and macrophages. Microb Pathog, DOI: PII: S0882-4010(12)00167-2.
Jezdimirović Milanka, Nevenka Aleksić, Ana Barać-Bašević, M Žutić, V Ivetić, O Radanović and N Jezdimirović, 2011. Comparative investigation on the efficacy of tulathromycin and florfenicol in the treatment of bronchopneumonia in feedlot calves Vet Glas, 65: 153-178. DOI: 10.2298/VETGL1104153J
Kurćubić V, 1993. Serological examinations of bovine viral diarrhea virus infection in cattle. Master Thesis, Department for microbiology and immunology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade.
Kurćubić V, R Đoković and S Jevtić, 2000. Five-years analyses of bacterial micro flora isolated from nasal swabs fattening calves and bullocks with respiratory syndrome-possibilities for prevention and therapy. Winter School for Agronomists, University in Kragujevac, Faculty of Agronomy in Čačak, 4: 9-16.
Kurćubić V, Z Ilić, R Djoković and S Jevtić, 2009. Identification of Cattle Persistently Infected (PI) with the bovine viral diarrhea virus (BVDV) using a screening method for a control and eradication program. Acta Agric Serb, 14: 79-92.
Kurćubić V, Z Ilić, R Djoković, S Jevtić and T Petrović, 2010a. Determination of the presence and degree of infection with bovine viral diarrhea virus in cattle herds in central Serbia - possibilities for control. Vet Glas, 64: 3-19. DOI:10.2298/VETGL1002003K
Kurćubić V, T Petrović, R Djoković, Z Ilić, 2010b. Investigation of bovine viral diarrhea virus (BVDV) and border disease virus (BDV) infections in different categories of sheep in Southwestern Serbia. Vet Glas, 64: 319-337.
Kurćubić VS, N Milić, RD Djoković and ZZ Ilić, 2011a. Evaluation of immunogenic properties of monovalent and polyvalent inactivated bovine virus diarrhea virus (BVDV) vaccines. Afr J Microbiol Res, 5: 2422-2427.
Kurćubić V, T Petrović, R Đoković, Z Ilić and MD Petrović, 2011b. Antibody response of beef calves to experimental monovalent and multivalent inactivated bovine viral diarrhoea virus vaccines as measured by indirect ELISA method. Biotech Anim Husbandry, 27: 901-991.
Kurćubić VS, RD Đoković, D Vidanović, M Šekler, K Matović, Z Ilić and J Stojković, 2013. Bovine respiratory disease complex (BRDC): viral and bacterial pathogens in Serbia. Biotech Anim Husbandry, 29: 29-36.
Larson RL and DL Step, 2012. Evidence-based effectiveness of vaccination against *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in feedlot cattle for mitigating the incidence and effect of bovine respiratory disease complex. Vet Clin N Am-Food A, 28: 97-106.
Lazić S, R Pavlović, M Lalić, S Đurišić and M Jovičin, 1995. Prevalence of infection caused by bovine herpesvirus-1 in cattle herds stem in vojvodina between 1992 and 1993. Vet Glas, 49: 99-103.
Lazić S, Ružica Ašanin, M Šajgalik, Maja Gagić, Branka Vidić and Dubravka Milanov, 2001. Effects of vaccination of highly pregnant cows with anti BHV-1 vaccines: 2, humoral response on the vaccine "Borinak". Acta Vet-Beograd, 51: 27-34.
Lazić S, T Petrović, Diana Lupulović and M Jovičin, 2003. Importance of latent IBR viral infection in cattle and possibilities for its eradication. Vet Glas, 57: 463-472.
Lazić Sava, Tamaš Petrović, Ivan Pušić and Maja Velhner, 2004. Most frequent calf diseases in industrial breeding. Vet Glas, 58: 67-76. DOI: 10.2298/VETGL0402067L
Lazić S, T Petrović, D Lupulović, D Bugarski, I Pušić, V Polaček and M Maljković, 2008. Prevalence of herpesvirus 1 in small herds in Southern Backa and Srem district. Arh Vet Med, 1: 16-29.
Lazić S, T Petrović, D Bugarski and N Kendrišić, 2009. Complex of respiratory diseases in cattle from the aspect of parainfluenza-3 virus. Biotech Anim Husbandry, 25: 703-711.

Loneragan GH, DA Dargatz, PS Morley and M Smith, 2001. Trends in mortality ratios among cattle in US feedlots. JAVMA, 219: 1122-1127.

Loneragan GH, DU Thomson, DL Montgomery, GL Mason and RL Larson, 2005. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. JAVMA, 226: 595-601.

Luo Y, Y Yuan, RG Ankenbauer, LD Nelson, SB Witte, JA Jackson and SK Welch, 2012. Construction of chimeric bovine viral diarrhea viruses containing glycoprotein E RNS of heterologous pestiviruses and evaluation of the chimeras as potential marker vaccines against BVDV. Vaccine, 30: 3843-3848.

Mahmood F, A Khan, MZ Khan, R Hussain, ST Gul and AB Siddique, 2012. Pathological and molecular based study of naturally occurring lentivirus infection. Pak Vet J, 32: 511-514.

Marinković D, Sanja Aleksić-Kovačević and P Plamenac, 2007. Cellular basis of chronic obstructive pulmonary disease in horses. Int Rev of Cytol, 257: 213-247.

Maunsell FP, AR Woolums, D Francoz, RF Rosenbusch, DL Step, DJ Wilson and ED Janzen, 2011. *Mycoplasma bovis* infections in cattle. J Vet Intern Med, 25: 772-83.

Milić N, Gordana Gadjanski-Omerović, Ružica Ašanin, J Nišavić and Marina Radojičić, 2003. Examination of antigenic structure and some biological activities of hem agglutinin-neuraminidase (HN) and fusion (F) glycoprotein antigens of Parainfluenza 3 virus, *in vitro*. Acta Vet-Beograd, 53: 321-331.

Nišavić J, N Milić, Aleksandra Knežević and Tanja Jovanović, 2010. The application of polymerase chain reaction in detection of bovine herpesvirus 1 in clinical samples. Acta Vet-Beograd, 60: 39-48. DOI: 10.2298/AVB1001039N

Pardon B, K De Bleecker, J Dewulf, J Callens, F Boyen, B Catry and P Deprez, 2011. Prevalence of respiratory pathogens in diseased, non-vaccinated, routinely medicated veal calves. Vet Rec, 169: 278.

Peterhans S, C Bachofen, H Stalder and M Schweizer, 2010. Cytopathic bovine viral diarrhea viruses (BVDV): emerging pestiviruses doomed to extinction. Vet Res, 41: 44-58.

Petrović T, 2002. Estimation of the prevalence of the infection in cattle caused by bovine viral diarrhoea virus. Master thesis, Department for infectious diseases and honey bees diseases, Faculty of Veterinary Medicine, University of Belgrade, Belgrade.

Petrović T, Bosiljka Đuričić, I Toplak, S Lazić, Darja Barlić Maganja, P Hostnik, J Grom and T Sandvik, 2004. Isolation and confirmation of bovine viral diarrhoea virus in Serbia and comparative typing with recent Slovenian isolates. Acta Vet-Beograd, 54: 33-42.

Petrović T, 2006. Identification and genetic characterization of BVD virus isolates on the territory of Republic of Serbia. PhD Thesis, Department for infectious diseases and honey bees diseases, Faculty of Veterinary Medicine, University of Belgrade.

Portis E, C Lindeman, L Johansen and G Stoltman, 2012. A ten-year (2000-2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex-*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in the United States and Canada. J Vet Diagn Invest, 24: 932-44.

Quinting B, B Robert, C Letellier, M Boxum, P Kerkhofs, F Schynts and A Collard, 2007. Development of a 1-Step Enzyme - Linked immunosorbent assay for the rapid diagnosis of bovine respiratory syncytial virus in postmortem specimens. J Vet Diagn Invest, 19: 283-43.

Raaperi K, S Bougeard, A Aleksejev, T Orro and A Viltrop, 2012. Association of herd BRSV and BHV-1 seroprevalence with respiratory disease and reproductive performance in adult dairy cattle. Acta Vet Scand, 54: 4.

Raue R, SS Harmeyer and IA Nanjiani, 2011. Antibody responses to inactivated vaccines and natural infection in cattle using bovine viral diarrhoea virus ELISA kits: assessment of potential to differentiate infected and vaccinated animals. Vet J, 187: 330-334.

Ridpath JF and RW Fulton, 2009. Knowledge gaps impacting the development of BVDV control programs in the United States. JAVMA, 235: 1171-1179.

Rogan D, S Lazić, R Pavlović and H Šamanc, 2010. Immunoprophylaxis of bovine respiratory syndrome. Vet Glas, 64: 393-408. DOI: 10.2298/VETGL1006393R

Schneider MJ, RG Tait, WD Busby Jr and JM Reecy, 2009. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. J Anim Sci, 87: 1821-1827.

Šamanc H, N Milić, V Stojić, D Knežević, I Vujanac, B Dimitrijević, J Nišavić and Marina Radojičić, 2009. Establishing presence of antibodies against bovine respiratory syncytial virus (BRSV), parainfluenza virus 3 (PI3) and bovine herpesvirus 1 (BHV 1) in blood serum of cattle using indirect immunoenzyme probe. Vet Glas, 63: 145-152. DOI:10.2298/VETGL0904145S

Takiuchi E, KC Medici, AF Alfieri and AA Alfieri, 2005. Bovine herpesvirus type 1 abortions detected by semi - nested PCR in Brazilian cattle herds. Res Vet Sci , 79: 85-88.

Taylor JD, RW Fulton, TW Lehenbauer, DL Step and AW Confer, 2010. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can Vet J, 51: 1095-1102.

Thonur L, M Maley, J Gilray, T Crook, E Laming, D Turnbull, M Nath and K Willoughby, 2012. One-step Multiplex Real Time RT-PCR for the detection of bovine respiratory syncytial virus, bovine herpesvirus 1 and bovine parainfluenza virus 3. BMC Vet Res, 8: 37. Available at: http://www.biomedcentral.com/1746-6148/8/37.

Urban-Chmiel R and DL Grooms, 2012. Prevention and control of bovine respiratory disease. J Livest Sci, 3: 27-36.

Wegelt A, I Reimann, H Granzow and M Beer, 2011. Characterization and purification of recombinant bovine viral diarrhea virus particles with epitope-tagged envelope proteins. J Gen Virol, 92: 1352-1357.

Wildman BK, T Perrett, SM Abutarbush, PT Guichon, TJ Pittman, CW Booker, OC Schunicht RK Fenton and GK Jim, 2008. A comparison of 2 vaccination programs in feedlot calves at ultra-high risk of developing undifferentiated fever/bovine respiratory disease. Can Vet J, 49: 463-472.

Zahur AB, U Farooq, M Hussain., SH Hashmi and R Muneer, 2007. Respiratory syndrome: A major threat to the livestock farmers and its economic impact. Pak Vet J, 27: 189-193.