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LABORATORY DIAGNOSTICS OF BOVINE PARAINFLUENZA-3 VIRUS, BOVINE HERPESVIRUS 1, AND BOVINE RESPIRATORY SYNCYTIAL VIRUS ASSOCIATED WITH BOVINE RESPIRATORY DISEASE

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Review paper

Abstract: The bovine respiratory disease complex (BRDC) is multifactorial and results from interactions between host factors, environmental factors, and pathogens. A virus, as an initial pathogen alters the animal's immunity supporting the bacterial colonization of the lower respiratory tract. Bovine herpesvirus 1 (BHV-1), bovine parainfluenza virus 3 (BPIV-3), and bovine respiratory syncytial virus (BRSV) are among the most significant viruses associated with BRDC. The disease most often affects young and older immunosuppressed animals. Laboratory results depend on the selected sampling site of the respiratory tract and proper timing during the period of virus shedding. The samples for testing mostly include nasal or nasopharyngeal swabs, tracheal wash, bronchoalveolar lavage fluid, or necropsy specimens. Virus isolation, although considered as the gold standard, is time-consuming and depends on the virus species and sampling conditions. Most of the virus identification methods used today are molecular assays (conventional and real-time PCR or RT-PCR) that are rapid, sensitive, and specific, which is of the essence in veterinary diagnostic laboratories. DNA sequencing is mostly used to detect specific genetic mutations and for molecular epidemiology of disease outbreaks. Serological diagnosis is performed based on the detection of specific antibody presence after infection of seronegative animals or a 4-fold specific antibody titer rise in paired serum samples. Different assays are available, including virus neutralization, complement fixation, haemagglutination inhibition, and ELISA. The early and reliable diagnosis is beneficial in the management and control of BRDC and is the basis of a timely treatment and prevention program.

Key words: BRDC, BHV-1, BPIV-3, BRSV, laboratory diagnosis

Introduction

Respiratory diseases lead to significant economic losses in cattle production and cause increased morbidity and mortality, with negative long-term consequences for herd health and productivity, including reproductive disorders, reduced milk production, and shortened lifespan. The disease prevalence increases in a stressful, overcrowded environment, and suboptimal environmental conditions (*Ellis, 2001; Van Der Fels-Klerx et al., 2002*). The bovine respiratory disease complex (BRDC) is multifactorial and is a consequence of different interactions between host factors, environmental factors, and pathogens. An initial pathogen alters the animal's immune mechanisms, allowing colonization of the lower respiratory tract by bacteria (*Ellis, 2009*). The BRDC can be caused by one or several primary pathogens including *Mycoplasma* species and respiratory viruses, followed by a secondary bacterial infection, or in some cases by bacteria unassisted. Some of the most common viral agents include the bovine respiratory syncytial virus (BRSV), bovine parainfluenza-3 virus (BPIV-3), and bovine herpesvirus 1 (BHV-1). In some cases, viruses can cause the appearance of clinical symptoms without bacterial superinfection, still, their role in the development of the clinical picture of respiratory diseases in cattle is mainly cofactorial (*Van Der Fels-Klerx et al., 2002; Ellis, 2009; Oliveira et al., 2020*). The bovine respiratory syndrome is a disease that most often affects young animals as well as older immunosuppressed individuals (*Ellis, 2001; Gershwin, 2012; von Messling, 2016*). The interpretation of laboratory test results considerably depends on the selected sampling site of the respiratory tract. Nasal swabs are frequently used as this sampling method is quick and uncomplicated, especially in large cattle populations (*Veljović et al., 2016; Nišavić et al., 2018a*). However, these are may not be applicable, especially for pathogens that replicate in the lower parts of the respiratory tract (*Ellis, 2009; von Messling, 2016; Kamdi et al., 2020*). Moreover, proper timing is of the essence and sampling should be performed during the period of virus shedding (*Grissett et al., 2015*). Nasopharyngeal swabs are usually more relevant, though the retrieved samples often contain many different microorganisms, sometimes affecting proper diagnosis. On the other hand, bronchoalveolar lavage is performed by endoscopy and can be meaningful in specific situations, e.g. when targetting the affected lung lobe (*Oliveira et al., 2020; Pardon and Buczinski, 2020*). A variety of laboratory tests can be used to identify respiratory viruses in field specimens collected during respiratory outbreaks in cattle. Virus isolation in cell culture and subsequent identification by neutralizing antibodies or immunofluorescence is often arduous and time

consuming for veterinary diagnostic laboratories, especially when timely diagnosis is of the essence (*Valarcher and Taylor, 2007; Milić et al., 2010; von Messling, 2016; Leme et al., 2020*). Serologic methods such as the virus neutralization (VN) test, complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), etc. are useful to evaluate infection dynamics or to determine the protective status of vaccination. However, these methods are not appropriate when the objective is to implement adequate therapy (*Šamanc et al., 2009; Sibhat et al., 2018; Pardon and Buczinski, 2020*). Today, most of the methods for virus identification rely on molecular assays based on polymerase chain reaction (conventional and real-time PCR or RT-PCR), thus adding to the speed, sensitivity, and specificity of virological diagnostics (*Boxus et al., 2005; Brodersen, 2010;Veljović et al., 2016; OIE, 2018*). The methods of DNA sequencing are mostly used for the detailed examination of the identified virus strains such as the detection of specific genetic mutations and molecular epidemiology of disease outbreaks, i.e. tracing the infection source and viral strain distribution (*Veljović et al., 2016; Krešić et al., 2018; Nišavić et al., 2018a; Nišavić et al. 2018b, Leme et al., 2020*).

Bovine parainfleunza 3 virus (BPIV-3)

Bovine parainfluenza-3 virus (BPIV-3) belongs to the genus *Respirovirus* and the family *Paramyxoviridae*. The viral genome consists of negatively oriented, unsegmented, and single-stranded RNA encoding six structural proteins: nucleocapsid protein, phosphoprotein, matrix (M) protein, fusion (F) protein, hemagglutinin-neuraminidase (HN) protein, and large (L) polymerase protein. The HN protein of the outer viral envelope is crucial for binding to cell surface receptors, while the F protein allows the fusion to the cell membrane, followed by the entry of the nucleocapsid into the host cell. Protein M interacts with surface glycoproteins HN and F and directs their insertion and aggregation at specific sites in the cell membrane (*Nišavić et al., 2006a; Nišavić et al., 2006b*; *Ellis, 2010; Veljović et al., 2016; Sobhy et al., 2017; ICTV, 2020*). This virus causes respiratory disease in cattle, and the clinical symptoms are manifested by anorexia, cough, fever, shortness of breath, and diarrhea in some cases. The bovine parainfluenza-3 virus has an immunosuppressive effect and causes bronchial pneumonia, which often occurs as a consequence of the primary viral and secondary bacterial infection. As previously stated, nonspecific environmental factors are a prerequisite for disease development (*Ellis, 2001; Ellis, 2010; Sobhy et al., 2017*). To date, three genotypes of parainfluenza-3 virus have been described, namely A, B and C. Genotype A was first described in the United States, and genotypes B and C in Australia and China (*Zhu et al., 2011; Oem et al., 2013*). The identification of new BPIV-3 genotypes is significant in view of the improvement of diagnostic methods and vaccine production (*Oem et al., 2013*).

Diagnosis of a BPIV-3 infection is based on the detection of live virus, viral antigen, or viral nucleic acid directly from animal samples or on the increase in specific antibody titers in paired serum samples. Samples for testing include nasopharyngeal swabs, tracheal wash, bronchoalveolar lavage fluid, or necropsy specimens (*Veljovic et al., 2016; von Messling, 2016; Oliveira et al., 2020*). The bovine parainfluenza-3 virus can be successfully isolated in cell lines of bovine origin such as bovine turbinate cells, or Madin-Darby bovine kidney cells (MDBK) (*Ellis, 2010; Veljović et al., 2016; Sobhy et al., 2017*). Identification of the virus in inoculated cell lines is based on the appearance of cytopathic effect (CPE), hemadsorption, or immunofluorescence staining (*Ellis, 2010; von Messling, 2016*). Viral RNA can be detected in suspected samples by RT-PCR or real-time RT-PCR (*Veljović et al., 2016; von Messling, 2016; Oliveira et al., 2020*). Usually, the BPIV3 shedding lasts for 1 to 2 days, the shedding peak occurs at day 4, and it mostly ceases in the first 10 to 13 days after infection (*Grissett et al., 2015*). In most cases, BPIV-3 is only detected during the first days after infection and is often absent when specimens are taken during secondary bacterial infections. Therefore, the detection of specific anti-BPIV-3 antibodies in paired serum samples can be performed by complement fixation, haemagglutination inhibition (HI), virus neutralization, or ELISA (*Ellis, 2010; von Messling, 2016*). The positive results of virological diagnostic tests should always be reviewed considering the high incidence of subclinical BPIV-3 infections and the multifactorial etiology of BRDC. Accordingly, the clinical condition of the herd and the sampled animal should always be assessed in parallel with laboratory results (*von Messling, 2016*). *Sobhy et al.* (*2017*) collected nasal swabs from 12 calves with clinical symptoms of pneumonia in Egypt and examined the presence of BPIV-3, BVDV, BRSV, and BHV-1 using the virus isolation method in MDBK cell line and the RT-PCR method. The presence of BPIV-3 was determined in eight samples, while the obtained results of the phylogenetic analysis showed that the Egyptian strains belong to genotype A. Additionally, the authors confirmed a higher prevalence of BPIV-3 in cattle compared to buffaloes. *Oliveira et al.* (*2020*) collected and examined 21 samples of bronchoalveolar lavage from 15 cows with clinical symptoms of respiratory infection, and 6 samples originating from asymptomatic cows. In 85.7% of the examined samples, the authors confirmed the presence of at least one causative agent, mixed infection was found in 72.2% of samples, while individual infection caused by only one pathogen was found in 27.7% of samples. Still, the presence of BHV-1, BPIV-3, and the bacterium *Mannheimia haemolytica* was not detected. In the studies of *Veljović et al.* (*2016*) performed in Serbia, 119 samples of nasal swabs were collected from cattle with clinical symptoms of respiratory infection and tested for the presence of BPIV-3 using virus isolation in the MDBK cell line and the RT-PCR method. The virus was successfully isolated from eight examined samples, and the identification was performed by the virus-neutralization test. All BPIV-3 strains detected in Serbia were assigned to genotype

C. Furthermore, the Serbian isolates showed a high degree of similarity with Chinese SD0805, SD0809, and SD0835 isolates, followed by isolates originating from South Korea, Japan, and the USA (TVMDL16 and TVMDL20). Concurrently, the Serbian strains differed from the BPIV-3 genotype B strains originating from Australia and the USA (TVMDL15 and TVMDL17). *Veljović et al.* (*2014*) examined 20 samples of bovine nasal swabs for the presence of BPIV-3 using virus isolation in the MDBK cell line and the RT-PCR method with specific F gene primers. The virus was identified in 4 samples, while F sequencing results demonstrated a high degree of similarity with analogous BPIV-3 sequences isolated in China and South Korea. Similarly, *Oem et al.* (*2013*) collected bovine nasal swabs from diseased animals with respiratory symptoms in South Korea. A total of five samples of bovine nasal swabs were examined using virus isolation, RT-PCR, and electron microscopy. The newly identified BPIV-3 strain differed from viruses belonging to genotypes A and B and was closely related to the Chinese SD0835 virus strain. Furthermore, the phylogenetic analysis revealed that the South Korean isolates belong to the C genotype indicating a correlation between the genetic variations in the viral genome and the geographical localization of isolates. In their tests, *Šamanc et al.* (*2009*) collected blood serum samples from unvaccinated cattle from nine farms in Serbia. A total of 92 samples were examined for the presence of antibodies against BRSV, BPIV-3, and BHV-1 using iELISA. Their results confirmed the presence of antibodies against BPIV-3, BRSV, and BHV-1 in 83.69%, 50%, and 20.65% of the samples, respectively.

Bovine herpesvirus 1 (BHV-1)

Bovine herpesvirus 1 (BHV-1) belongs to the genus *Varicellovirus*, the subfamily *Alphaherpesvirina*e, and the *Herpesviridae* family. It is a double-stranded DNA virus with an outer envelope containing glycoprotein antigens crucial for viral binding to cell surface receptors and the process of viral replication (*Osterrieder, 2016; ICTV, 2020*). BHV-1 causes infectious bovine rhinotracheitis (IBR), manifested by upper respiratory tract disease, conjunctivitis, and bronchopneumonia. The appearance of erosions and ulcers in the upper respiratory tract, mostly the trachea, are characteristic of BHV-1 infection (*Ellis, 2009*). Another infection form caused by this virus is known as infectious pustular vulvovaginitis, manifesting as inflammation of the vulva and vagina, balanoposthitis, and abortion (*Muylkens et al., 2007*). After transmission which usually occurs by direct contact, BHV-1 infects the epithelial cells of the upper airways, as well as nerves (*Ellis, 2009*). Like other herpesviruses, BHV-1 causes a lifelong latent infection with periodic virus reactivation from the neural tissue and shedding caused by stress and immunosuppression. Bovine herpesvirus 1 can trigger BRDC through immunosuppression that enables secondary infections leading to pneumonia and sometimes death (*Muylkens et al. 2007; Ellis, 2009;*

Osterrieder, 2016; Jones, 2020). This virus causes lysis of the ciliated epithelium of the trachea which contributes to the aggregation of bacteria in the upper airways, thereby resulting in pneumonia. Moreover, BHV-1 downregulates type 1 interferon, induces the apoptosis of CD4+ T cells, and reduces MHC I expression (*Ellis, 2009*). The average time for BHV-1 shedding is 2 days, while peak shedding occurs on day 4 post infection (*Grissett et al., 2015*).

This infection is mostly diagnosed by virus isolation in cell culture, serology, and PCR. BHV-1 can be isolated from nasal swabs, conjunctival swabs, tonsils, and lungs using cells of bovine origin or Vero cells, characterized by the appearance of rapid cytopathic effect with the formation of syncytia (*Nišavić et al., 2010; Biswas et al., 2013; Osterrieder, 2016*). The virus is further identified by virus neutralization test using BHV-1 antiserum or by direct detection of BHV-1 antigen by immunofluorescence (*OIE, 2018*). Specific anti-BHV-1 antibodies are detected in the serum of cattle within 2–3 weeks of infection (Graham et al., 1997; Biswas et al., 2013). Usually, the BHV-1 seroconversion takes place around day 18, reaching the peak on day 40 after infection (*Grissett et al., 2015*). The indirect and blocking ELISA are used more extensively than the virus neutralization test due to its suitability for screening large numbers of samples in a shorter period. It is usually necessary to test paired serum samples collected from animals suspected of BHV-1 infection (*Graham et al., 1997; OIE, 2018; Sibhat et al., 2018*). The polymerase chain reaction, especially the real-time PCR, is now routinely used for the detection of viral DNA in examined samples as a rapid and reliable method (*Milić et al., 2010; Biswas et al., 2013; OIE, 2018; Nišavić et al., 2018a; Oliveira et al., 2020*).

Milić et al. (*2010*) collected 65 samples of nasal swabs of calves and heifers from several farms in the Republic of Serbia. The samples were examined for the presence of BHV-1 using virus isolation and PCR with primers for thymidine kinase and glycoprotein B (gB) and real-time PCR with gB specific primers. Virus isolation in cell culture was unsuccessful, however, the use of conventional PCR and real-time PCR yielded 1 and 3 positive samples, respectively, justifying the use of molecular methods in the diagnostics of BHV-1 infection. Furthermore, 20 bovine nasal swab samples were examined for the presence of BHV-1 using the standard method of virus isolation and PCR, and accordingly, the virus was detected solely by molecular methods (*Nišavić et al., 2010*). Moreover, *Nišavić et al.* (*2018a*) collected 110 nasal swabs and determined the presence of BHV-1 in 4 samples by both virus isolation and PCR. Phylogenetic analysis of the gB gene of these Serbian isolates showed 100% similarity with analog BHV-1 sequences from Egypt and the USA, and 99% to 98% with BHV-1 strains from Israel, India, Brazil, and the USA. The analysis of the thymidine kinase encoding gene grouped Serbian BHV-1 strains with virus isolates from the USA and Australia. Multiplex PCR has proved to be a reliable and convenient method for simultaneous detection and differentiation between BHV-1 and BHV-5

using specific primers for glycoprotein C (gC) (*Claus et al., 2005*). Bovine herpesvirus 1 is divided into two genotypes, namely 1.1 and 1.2, and genotype 1.2 is further separated into BHV-1.2a and 1.2b. *Zhou et al.* (*2020*) examined 102 lung samples originating from calves that died with symptoms of respiratory infection. Samples were collected from animals originating from 13 different bovine populations in China. BHV-1 was isolated, and the selected isolates were phylogenetically analyzed using gC primers. The obtained results showed that all isolates belong to the BHV-1.2b gene subtype, which appears to be dominant in China. The molecular analysis of the gC encoding region of bovine herpesvirus isolates originating from Brazil, Uruguay, and Argentina was performed (*Traesel et al., 2013*). This study showed that the gene sequence encoding the viral glycoprotein C is suitable for phylogenetic analysis of BHV-1 and BHV-5 virus strains and confirmed a clear difference between BHV-1 and BHV-5 viruses as well as between BHV-1 virus subtypes (BHV-1.1 and BHV-1.2). BHV-1 infection monitoring programs are based on the detection of the presence of the virus and the differentiation between vaccinated and infected animals. Vaccination of cattle is performed with a vaccine virus strain devoid of glycoprotein E. Accordingly, *Wernike et al.* (*2011*) developed a highly sensitive triplex real-time PCR method that enables the differentiation of field and vaccine BHV-1 strains. The BHV-1 seroprevalence was examined recently in 1,379 dairy cows from 149 cattle populations in Ethiopia (*Sibhat et al., 2018*). The collected milk samples were tested using ELISA, which proved to be a convenient method, and all the cattle populations had an average level of BHV-1 seroprevalence of 81.8%.

Bovine respiratory synctytial virus (BRSV)

Bovine respiratory syncytial virus (BRSV) belongs to the genus *Orthopneumovirus* which belongs to the *Pneumoviridae* family. The virus possesses a single-stranded 15 kb RNA molecule and has an irregular virion shape ranging from almost spherical to filamentous. The viral envelope contains three significant glycoproteins: glycoprotein G that enables the binding to the host cell surface, fusion protein (F), and small hydrophobic (SH) protein (*von Messling, 2016; ICTV, 2020*). The fusion protein leads to the fusion of cell membranes with the consequent formation of syncytia, thus facilitating the virus movement from cell to cell. These three glycoproteins are responsible for protective immunity development (*Valarcher and Taylor, 2007; Gershwin, 2012*). The genetic variations of up to 11%, mostly in the G protein are a consequence of viral RNA-dependent RNA polymerase and the absence of exonuclease proofreading (*Larsen et al., 2000*). The BRSV G protein is often used in studies concerning the classification of this virus considering its genetic and antigenic heterogeneity (*Valarcher et al., 2000*). The bovine respiratory syncytial virus has been classified into four antigenic subgroups, A, B, AB, and untyped based on their G protein

antigenic differences, however, this association has unknown biological implications (*Valarcher and Taylor, 2007*). Based on the genetic differences od G and F encoding sequences, BRSV strains are currently divided into subgroups I to VIII (*Leme et al., 2020*). Infection caused by BRSV occurs in all age groups, but the disease most often affects recently weaned calves and young cattle, causing pneumonia and predisposing them to other respiratory infections. Clinical symptoms include fever, anorexia, depression, dyspnoea, mucous nasal discharge, while the so-called 'air-hunger' position of mouth breathing with the head and neck outstretched is also present in severe cases (*Gershwin, 2012; von Messling, 2016; Leme et al., 2020*). Cattle are the natural hosts of BRSV, however, it is possible that small ruminants also play a role in virus transmission (*von Messling, 2016*). This virus is mainly transmitted through direct contact or by aerosol and it replicates in the superficial layer of the respiratory ciliated epithelium and replication can also be detected in type II pneumocytes (*Valarcher and Taylor, 2007; von Messling, 2016*). Bovine respiratory syncytial virus infection contributes to the development of secondary bronchopneumonia since bacteria deposit in the lower respiratory tract as a result of the damage to the mucociliary escalator function (*Ellis, 2009*). The shedding of BRSV can usually be detected on day 3 postinfection with the average time to peak on day 5, and time to resolution between days 7–14 (*Grissett et al., 2015*).

The diagnosis of BRSV is rarely based on the technique of virus isolation since little or no cytopathic effects are seen following inoculation to cell lines of bovine origin. The virus can be detected by immunofluorescence directly in the sampled material such as tracheal wash, nasopharyngeal swabs, tissue samples, or in inoculated cell lines (*Valarcher and Taylor, 2007; von Messling, 2016; Leme et al., 2020*). However, immunofluorescence results depend on the sampling moment and have limitations when examining field specimens due to tissue autolysis or the appearance of non-specific fluorescence (*Brodersen, 2010*). The recent study of *Kamdi et al.* (*2020*) describes the examination of samples collected from dead cattle and buffaloes under 12 months of age in India. The viruses detected by RT-PCR included BPIV-3 and BRSV, and this finding was confirmed by PCR product sequencing and direct immunofluorescence. Necrosis of the lung epithelium, thickening of the alveolar septa, and filling of the alveolar lumen with mononuclear cells and syncytial cell formations was evident in BRSV-positive lung samples. RT-PCR tests are most often used for routine diagnostic purposes, having the advantage of not being affected by the presence of neutralizing antibodies in the sampled material (*von Messling, 2016*). Accordingly, RT-PCR detects BRSV in the specimens of nasal secretions for a longer period compared to ELISA tests that are also limited by the rising titers of specific anti-BRSV antibodies (*Brodersen, 2010*). The RT-PCR method specific for the viral G protein is suitable for BRSV identification in the samples of lungs and tracheal tissue from calves with mild to severe interstitial pneumonia (*Almeida et al., 2005*). Real-time RT-PCR with

specific primers for the highly conserved viral nucleoprotein was 100 times more sensitive than the conventional RT-PCR method in the detection of BRSV in lung, trachea, and bronchoalveolar fluid samples from cattle exhibiting clinical symptoms of respiratory disease (*Boxus et al., 2005*). Still, the possible detection of viral nucleic acid from vaccinal viruses must always be considered (*Brodersen, 2010, von Messling, 2016*). *Leme et al.* (*2020*) performed the molecular characterization of G and F proteins of Brazilian BRSV field strains isolated from respiratory disease cases in 10 different cattle populations. Two strains had a high degree of similarity with analogous sequences from subgroup III, including the Bayovac vaccine strain, while the remaining Brazilian BRSV strains were different and represent a probable new virus subgroup with a mutation in the immunodominant region of the G protein. The F gene of the Brazilian viruses also bears mutations that do not exist on the F gene of other BRSV strains from the subgroups known thus far. *Krešić et al.* (*2018*) examined Croatian BRSV strains detected in the samples of nasal swabs, blood, lungs, and lymph nodes from one to six-month-old calves originating from ten cattle populations in different districts. The presence of viral RNA was not detected only in the examined blood samples. Sequencing analysis of selected BRSV strains was performed and the phylogenetic analysis showed clustering within three different genetic subgroups, namely II, VII, and VIII. These authors also revealed unique mutations within an essential immunodominant region of the viruses from subgroups II and VII. Serologic diagnosis is performed based on the detection of a 4-fold titer rise in paired serum samples, and different assays are available including virus neutralization, complement fixation, and ELISA (*Graham et al., 1997; von Messling, 2016*). Average time to BRSV seroconverison is 9 days (range 5–21 days), peaking on day 23 after infection (*Grissett et al., 2015*). *Roshtkhari et al.* (*2012*) determined the presence of BRDC-associated viruses through the examination of paired serum samples of calves with pneumonia using iELISA. During the testing period, 56.4% of blood serum samples were found to have antibodies against BHV-1, 81.5% against BRSV, 89.5% against BPIV-3. Seroconversion was found for BRSV in 16.6% and BPIV-3 in 26.1% samples, while it was not established for BHV-1.

The bovine respiratory syndrome represents a major cause of morbidity, mortality, and economic loss in farms around the world despite available therapy and immunoprophylaxis. With this in mind, appropriate and reliable laboratory diagnosis of this complex disease is very important to prevent the spread of pathogens in large animal populations. The sampling method must be properly assessed, paired with the time frame that coincides with virus shedding. Timely diagnosis is beneficial in managing and controlling BRDC, and early treatment of calves is the most important feature of a successful treatment program. It should be noted that the availability of many classical and molecular methods of laboratory

diagnostics for the identification of viruses involved in BRDC significantly contributes to the detection of infections discussed in this text.

Laboratorijska dijagnostika virusa parainfluence 3 goveda, goveđeg herpesvirusa 1 i goveđeg respiratornog sincicijalnog virusa kao uzročnika respiratornog sindroma goveda

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Rezime

Kompleks respiratornih bolesti goveda (BRDC) nastaje kao posledica interakcije između faktora vezanih za domaćina, faktora okoline i različitih mikroorganizama. Virus kao primarni patogen najčešće dovodi do supersije imunoloških mehanizama životinje i omogućuje kolonizaciju donjih disajnih puteva bakterijama. Goveđi herpesvirus 1 (BHV-1), virus parainfluence 3 goveda (BPIV-3) i goveđi respiratorni sincicijalni virus (BRSV) su među najznačajnijim uzročnicima BRDC, pri čemu najčešće oboljevaju mlade i starije imunokompromitovane jedinke. Rezultati laboratorijske dijagnostike zavise od mesta uzorkovanja materijala i od izbora trenutka za uzimanje uzorka u odnosu na dinamiku izlučivanja virusa u spoljašnju sredinu. Uzorke za ispitivanje uglavnom predstavljaju nosni ili nazofaringealni brisevi, trahealni aspirati, tečnost dobijena bronhoalveolarnom lavažom ili tkiva. Izolacija virusa, iako predstavlja zlatni standard, oduzima dosta vremena i njena uspešnost zavisi vrste virusa, načina uzorkovanja, odnosno stanja uzorka. Većina metoda identifikacije virusa koje se koriste u današnje vreme su molekularni testovi (konvencionalni i real-time PCR ili RT-PCR) koji se brzo izvode, osetljivi su i specifični, što je od suštinske važnosti u veterinarskim dijagnostičkim laboratorijama. Sekvenciranje virusne nukleinske kiseline se upotrebljava u cilju detekcije specifičnih mutacija u virusnom genomu i molekularno-epidemiološko praćenje kretenja i širenja pojedinih virusnih sojeva. Serološka dijagnostika se zasniva na pojavi specifičnih antitela kod serološki negativnih jedinki, odnosno detekciji četvorostrukog porasta titra specifičnih antitela u parnim uzorcima krvnog seruma, a dostupni su različiti testovi, uključujući metod virus-neutralizacije, reakciju vezivanja komplementa, inhibiciju hemaglutinacije i ELISA. Pravovremeni i pouzdani rezultati laboratorijske dijagnostike su važni u cilju kontrole BRDC i predstavljaju osnovu za blagovremeno sprovođenje terapije i programa prevencije širenja infekcije.

Ključne reči: BRDC, BHV-1, BPIV-3, BRSV, laboratorijska dijagnostika

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Author Contributions

JN and AR conceptualized the paper, which was developed further in discussion with NM, AS, and LjV. JN and AR collated articles for review, wrote and critically reviewed various drafts, NM, AS, and LjV contributed to the preparation of the final version and provided consent for submission.

Conflicts of Interest

The authors declare no conflicts of interest.

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