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Diagnostics of bovine respiratory disease: An updated review

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Abstract
Bovine respiratory disease is a 'disease of lower respiratory tract' of cattle or buffaloes having multifactorial etiology involving complex interaction of environmental stress factors, host factors and infectious agents. Early and accurate diagnosis of disease is very necessary as there are heavy economic losses due to this condition. Confirmation of respiratory disease can be done using variety of methods. Often, visual and physical examination has been done in the field, but this can lead to inaccurate diagnosis. Radiography and ultrasonography can be very handy in diagnosis as well as prognosis of this disease. Diagnostic sampling and tests can provide valuable information when investigating causes of respiratory disease. Tran's tracheal wash and bronchoalveolar lavage samples can provide samples from lower respiratory tract. Pathologic and laboratory investigations are necessary when a specific diagnosis of the cause is required. Causative bacterial and viral pathogens identification should be done by culturing the suitable sample by standard protocol and use of molecular diagnostic techniques *e.g.* polymerase chain reaction. Necropsy and diagnostic testing for BRD pathogens are the gold standard tests to diagnose BRD.

Keywords: bovine, diagnosis, methods, respiratory disease

1. Introduction

Year after year, diseases of the respiratory system are a major cause of illness and death in dairy cattle or buffaloes. Research on bovine respiratory disease (BRD) began in the late 1800's and still it is one of the most widely studied disease (Taylor *et al.*, 2010) [40]. Despite advances in veterinary medicine and animal husbandry, BRD remains a challenging issue for owners as well as veterinarians. Both short-term and long-term economic impacts has been associated with BRD on bovine production (Teixeira *et al*., 2017) [41]. It is very important disease of cattle globally with economic losses of more than 3 billion dollars per year (Watts and Sweeney, 2010) [46]; which includes the costs of treatment, prevention and production losses (Cernicchiaro *et al*., 2013) [10]. BRD morbidity, mortality, prevention and treatment results in economic loss estimated to be from 13.90 to 15.57 dollars per head (Snowder *et al*., 2007) [39]. It accounts for approximately 65-80% of morbidity and 45-75% of mortality in cattles (Duff and Galyean, 2007) [13].

The BRD complex is a disease of the lower respiratory tract, has a multifactorial etiology and resulting in bronchopneumonia due to complex interaction between environmental stress factors, host factors and infectious agents (Caswell, 2014; Guzman and Taylor, 2015) [8, 18]. Environmental factors like weaning, transport, commingling, bad weather and dust serve as stressors that adversely affect the immune and non-immune defence mechanisms of the host; while crowding and poor ventilation can enhance the transmission of infectious agents among animals. Pathogenesis typically consists of changes in respiratory mucosa on animal's immune dysfunction by viral infections which favours bacterial colonization and proliferation, creating secondary infections (Horwood *et al*., 2014) [20]

Infectious agents commonly identified are viruses like bovine herpesvirus type 1 (BHV-1), parainfluenza-3 virus (PI-3), bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV) (Grissett *et al*., 2015) and bovine coronavirus (BCV) (Caswell *et al*., 2012) [9]; bacteria such as *Mannheimia haemolytica* (Boukahil and Czuprynski, 2016) [4], *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis* (Grissett *et al.*, 2015) and *Trueperella pyogenes* (Caswell *et al*., 2012) [9]. Although there are some other respiratory disorders, such as acute bovine pulmonary oedema and emphysema, allergic reactions, lungworm, atypical interstitial pneumonia and calf diphtheria, BRD is commonly encloses pneumonia in cattle resulting in a complex range of pulmonary lesions (Guterbock, 2014) [17].

As disease is responsible for heavy economic losses, accurate and timely recognition is very important for its successful treatment. Current methods for diagnosing BRD are lacking usefulness and effectiveness (Miller, 2016) [27]. Developing other means of diagnostics must be established in order to reduce BRD occurrence. One of the important challenge faced by the dairy farmers or feedlot personnel is early and accurate identification of disease. Improved means of diagnosing this disease will allow animals to have less stress, lower morbidity and effective treatment which prevent disease dissemination in the herd along with enhanced animal welfare of cattle (Miller, 2016) [27]. To date, the systematic assessment of diagnostic test accuracy has not been commonly used in veterinary medicine and animal science (Lamb and Nelson, 2015) [23].

It is to be hoped that this article as reference may provide a diagnostic plan of bovine respiratory disease for the practicing veterinarians. Numerous strategies to diagnose this diseased condition are as follows:

2. History

Good clinical history can help the individual in making the list of differential diagnosis, selecting appropriate tests to make economic advantage as well as animal welfare and also suggestive of risk factors which ultimately helps in preventing disease to group of animals. Clinical information like age, duration of illness, environment or housing, type and source of feed, clinical signs and their sequence of onset, common factors among affected animals, change in manage mental practices, vaccination history and response to treatment administered should be included in history taking (Cooper and Brodersen, 2010) [11].

3. Clinical signs

Clinical signs observation is the most common method to identify the animal affected with BRD. Without using the expensive equipment's, clinical scoring system would be useful tool for farmers, clinicians and researchers. Total score is made by assigning the values to clinical signs in scoring systems (Love *et al*., 2014) [24]. Following table shows the clinical signs associated with BRD along with scores given according to their severity.

Table 1: Animal suspected to have BRD were scored using clinical scoring system (Mc Guirk, 2008)

Clinical signs	Score			
	0	1	2	3
Cough	None	Single induced	Multiple induced	Multiple spontaneous
Nasal discharge	None	Small amountnof unilateral cloudy discharge	Bilateral, cloudy, or excessive mucus discharge	Copious bilateral mucopurulent discharge
Ocular discharge	None	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Ear & Head carriage	Normal carriage	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
Rectal temperature (°F)	<100.9	101.0–101.9	102.0–102.9	>103.0

The lung inflammation leads to occurrence of clinical signs. Clinical signs such as tachypnea, fever, dyspnoea, nasal discharge, inappetance and coughing are used as the primary form of detection of respiratory disease (Ozkanlar *et al*., 2012; Buczinski *et al.,* 2014; Toaff-Rosenstein and Tucker, 2018) [31, 6, 44]. Thoracic auscultation is a basic part of the analysis of the ruminant respiratory tract (Wilkins and Woolums, 2009; Mang *et al*, 2015) [48, 25]. Abnormal lung sounds, such as increased bronchial sounds (Ozkanlar *et al*., 2012; Berman *et al*, 2019) [31, 3], crackles (or rhonchi) and wheezes can be heard on thoracic auscultation in cases of pneumonia (Buczinski *et al.,* 2014; Mang *et al*, 2015) [6, 25]. Unfortunately, diagnostic accuracy of visual observation or clinical signs associated to this disease is poor (Thompson *et al*., 2006; Schneider *et al*., 2009; White and Renter, 2009; Portillo, 2014) [43, 37, 47].

4. Imaging: Radiography& Ultrasonography

Diagnostic imaging procedures like radiography and ultrasonography have been widely used in bovine medicine in the past decades. These are non-invasive methods for ante mortem diagnosis of pneumonia (Ollivett & Buczinski, 2016) [29]. However, one should expect to see lung consolidation, characteristic or pattern of pneumonia, abscesses and extra pulmonary air or fluid. There is tendency to misread bovine chest radiographs as abnormally dense and incorrectly conclude that the animal has pneumonia because normal bovine lung has a greater background density than the lungs of dogs and horses (Farrow, 1999) [14].

Use of radiography for the diagnosis of lung diseases in cattle is not practical (Siegrist and Geisbühler, 2011) [38] in farm conditions, because of equipment limitation, costs, anesthetic requirements and potential for radiation exposure; while now-a-days it had been replaced by ultrasonography (Braun, 2009) [5], because thoracic ultrasound can be performed as animal-side test using portable, readily available machines without the fear of exposure to radiation. Thoracic ultrasound detects the non-aerated or consolidated lung lesions, which change the ultrasonographic character of the lung from strong reflector with reverberation artifact to a homogeneous, hypoechoic structure similar to that of the liver (Reef *et al*., 1991) [35] and diagnose the pneumonia in any clinical state of animal (Ollivett *et al*., 2015) [30]. However, consolidation of lung is not associated with active lung infection. It can also be found in cases of lung infarction, atelectasis and fibrosis (Sartori and Tombesi, 2010) [36]. Tharwat and Oikawa (2011) [42] reported that bovine thoracic ultrasonography can be used as a screening tool and it allows assessment of the extent and severity of pulmonary changes.

5. Sampling and diagnostic tests

Diagnostic sampling and tests can provide valuable information when investigating causes of respiratory disease within a group of animals. Various ante-mortem and post-mortem methods for sampling from the respiratory tract of cattle for bacterial culture have been investigated and include nasal swab (NS), guarded nasopharyngeal swab (NPS), trans tracheal wash (TTW), Broncho alveolar lavage (BAL) (Doyle *et al*., 2017) [12] and tissue samples at necropsy (Urban-Chmiel and Grooms, 2012) [45] are commonly used sampling methods to identify the respiratory pathogens associated with BRD. Kumar *et al*., (2015; 2017) [21, 22] collected nasal swabs samples from affected buffaloes and identified the bacteria involved in the causation of BRD. Although each method had its own advantages and disadvantages, but TTW has been recommended for analysis of the microbiological status of the lower parts of bovine respiratory tract (Pommier and Wessel, 2002) [32] and can provide samples for a broader diagnostic approach than nasal or nasopharyngeal swabs (Cooper and Brodersen, 2010) [11] by avoiding the nasopharyngeal contamination. Broncho alveolar lavage is also a well-established diagnostic procedure which involves washing a sample of cells and secretions from the alveolar and bronchial airspaces. As well as, BAL is less invasive than a TTW and cattle generally tolerate BAL well without sedation (Capik *et al*., 2017) [7]. Narang (2017) [28] diagnosed the lower respiratory tract infection in cattle by conducting cytological examination, bacterial isolation, PCR assay (major pathogens) using tracheal wash and also by radiographic imaging technique. Disease can often be definitively diagnosed at necropsy. Diagnostic samples collected during post-mortem should be from animals preferably which were not treated in early stage of the disease. Select lung samples for histopathologic examination, immunohistochemistry or fluorescent antibody testing at the junction of affected and less affected tissues (Cooper & Brodersen, 2010) [11]. Presence of lung lesions is a common method of determining previous respiratory lung infections (Miller, 2016) [27]. This is followed by laboratory evaluation to identify the causal agent and inflammatory changes.

6. Lab evaluation

Samples collected from animal for culture should be in good condition with minimal contamination. Prolonged duration of post-mortem or gross contamination of samples prevents pathogens isolation as well as other testing procedures. It is recommended to keep samples cold but not in frozen state until reaching the laboratory. Trans-tracheal washes, bronchoalveolar lavages, nasopharyngeal swabs or nasal swabs samples should be collected from untreated animals soon after onset of the disease process (Cooper and Brodersen, 2010) [11]. These specimens should be kept chilled and submitted for culture.

Bacterial culture is important for confirming the presence of bacterial infection, identifying the responsible pathogen. Identification of cultured bacteria is based on characteristics of colony growth and appearance as well as biochemical testing of individual colonies (Quin *et al.*, 2002) [34]. Cytology of TTW can be a helpful diagnostic method which helps to differentiate inflammation, neoplasia and specific pathogens of lower respiratory tract (Hewson and Arroyo, 2015) [30, 19].

For identification and characterization of both viral and bacterial pathogens of BRD many useful laboratory methods are available including culture, immunohistochemistry (IHC), antigen capture ELISA, culture and PCR assays (Duff and Galyean, 2007) [13]. The bacterial infections involved in BRD can be diagnosed based on many species-specific methods such as conventional bacterial cultivation (Autio *et al.* 2007) [2], phenotyping characterization (Angen *et al.*, 2002) [1]. In addition, new diagnostic techniques used for the detection of BRD are virus neutralization test, *in-situ* hybridization, complement fixation test (CFT), agglutination test and multiplex PCR (Fulton and Confer, 2012) [15]. PCR based molecular diagnosis are widely used in veterinary medicine for BRD pathogens (Fulton and Confer, 2012).

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