**SUMMARY**

During the period of investigation (Oct. 2015 to Sept. 2022), a total number of 50 cattle in different areas of Sohag Governorate, South Egypt, were necropsied due to the bad-sequel of BRD. They were unvaccinated against respiratory infection and did not respond to the therapeutic trails by fieldveterinarians. Their lungs grossly described and culturally examined for the presence of Mycoplasma infection. Grossly, the affected lungs showed multiple areas of pulmonary sequestration in 41/50 (82.00%) of the tested cases with remarkable thickening and fibrosis of the interlobular septa. Caseonecrotic bronchopneumonia with bronchiectasis and the small airways filled by yellowish caseated purulent exudate was discerned. All culturally examined lungs (50 cases) were Mycoplasma positive. The majority (90 %) of the isolated Mycoplasma strains were biochemically glucose and arginine negative with production of film and spots. The PCR—tested strains were Mycoplasma bovis infection. The obtained results are strongly denotes Mycoplasma bovis is a major persistent pneumonic pathogen of BRD in Sohag Governorate and it is concluded that vaccination program against the most common pneumogens including Mycoplasma bovis should be regularly warranted.

**INTRODUCTION**

BRD is one of the most common problem deterring the productive and reproductive capacities of cattle herds (Decaris et al., 2022). BRD has been reported with variability from 5 to 66% in feedlot cattle and it is the most costly beef cattle disease (Snowder, 2009). BRD is a multifactorial syndrome. Stress factors, bad management in association with various pathogens are the major factors causing BRD in cattle herds (Sayed and Zaitoun, 2009, Taylor et al., 2010, Gaeta et al., 2018, El-Seedy et al., 2020 and Hashem et al., 2022).

Etiologically, several pathogens encountered as BRD's pathogens. Bacterial pathogens play a pivotal role in BRD (Zecchinon, and Desmecht, 2005). They emphasized that Mannheimia hemolytica and its leukotoxin suppress the defense mechanism of the infected cattle and other ruminants and induces a favorable chance for invasion of other pathogens. On the other side, Yates (1982) and Lopez (2001) corroborated that respiratory viruses particularly bovine herpes viruses and parainfluenza type3 were more prominent pathogens than bacteria in induction of BRD in bad managed herds. They added that the respiratory viruses damage the windpipe allowing bacteria enters the deep respiratory system of the infected cattle. Moreover, Fulton (2009) encountered Bovine Herpes Virus 1 (BHV1) was more prevalent pneumogenic agent rather than Bovine Virus diarrhea (BVDV) and Bovine Respiratory syncytial Virus (BRSV).

Concerning Mycoplasma infection, reviewing of the available literature reveals various types of Mycoplasma infection were encountered as a major
respiratory pathogen in large and small ruminants (Zaitoun, 2001; Nicholas, 2011; Kanci et al., 2017 and Hashem et al., 2022). However, Mycoplasma bovis is frequently incriminated as an outstanding primary pathogen responsible for BRD in large ruminants (Lysnyansky and Ayling, 2016 and Mahmood et al., 2017 and Hashem et al., 2022). In spite of the bacterial pathogens of calves’ respiratory affections in certain areas of the mid and Upper Egypt were elucidated by El-Seedy et al (2020), role of Mycoplasma in BRD in south Egypt is still scanty. However, Hashem et al (2022) declared that 13.33 % of the examined diseased calves (n = 60) with respiratory manifestations in Sadat City (Menoufiya Governorate, North of Egypt) was harbor Mycoplasma infection in their nasopharyngeal regions. Their results concluded that the rate of Mycoplasma bovis (8.33%) infection was more prominent than bacterial pathogens particularly Pasteurella multocida (5%) and Staphylococcus aureus (5%). The current situation of Mycoplasma infection in cattle with signs of BRD in the southern governorates of Egypt like Sohag Governorate appears to be scanty. Consequently, screened of Mycoplasma infection particularly Mycoplasma bovis in lungs tissues of the necropsied cattle showed severe pneumonias was aimed in the current work.

Material and Methods Ethics approval:

All procedures were carried out according to the experimental standards approved by the Animal Research Ethics Committee at Faculty of Veterinary Medicine, Sohag University.

Animal:

During the period of investigation (Oct. 2015 to Sept. 2022), a total number of 50 cattle in different areas of Sohag Governorate, South Egypt, were necropsied due to the bad-sequel of BRD. Based on history taking all cases were unvaccinated against respiratory infection and showed severe signs of BRD (score 3) according to Wisconsin and California scoring system (Decaris et al., 2022). Fifty cases were necropsied due bad sequel of BRD, and their lungs were grossly described and culturally examined for the presence of Mycoplasma infection.

Samples and laboratory procedures:

Tissues’ specimens of the pneumatic lungs of the necropsied cases were aseptically excised and immediately immersed in screw-capped bottles containing Mycoplasma broth culture supplemented with (growth enhancers for bovine Mycoplasmas, and bacterial inhibitors as prescribed previously by Zaitoun, 1990). The broths were incubated at 37 OC. Two days later, the incubated broths were repeatedly blindly subcultured in new broths and incubated. Three blind passages were carried out. Thereafter, the incubated broths were platted onto Mycoplasma agar medium and incubated in Gas-pack Jar with 10% co2 atmosphere for two days. Post incubation, the plats were regularly examined for one week. The characteristic colonies were picked-up and purified by further subculturing processes.

Biochemically, the purified colonies were subjected to Genus determination and biochemical characterizations (glucose fermentation, arginine deamination and Film and spots production tests) as approved by Stalheim (1990). The biochemically glucose negative and arginine negative strains with production of film and spots were molecularly identified using conventional PCR technique.

PCR technique using species-specific premiers for Mycoplasma bovis.(Table DNA extraction of the tested and control samples were carried out based on manufacturer of QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany, catalog no.: 511304). Forward and reserved sequences of PCR’s primer of both Mycoplasma bovis were illustrated onto Table 2. Protocol of PCR technique of the tested samples was carried out based on Kounour (2022) in The Biotechnology Unit of Faculty of Veterinary Medicine, Sohag University, Egypt.

RESULTS

Currently, multiple areas of pulmonary sequestration were grossly observed in the majority of the tested cases with dramatic thickening and fibrosis of the interlobular septa, (Fig. 1: A&B). Moreover, caseonecrotic bronchopneumonia with bronchiectasis and the small airways filled by yellowish caseated purulent exudate was noticed (Fig.2).

Mycoplasma infection of the tested cases (n = 50):

The cultural and biochemical characterizations of the tested samples indicated that all tested lungs’ specimens of severely infected cattle with BRD were Mycoplasma positive and 73 strains of Mycoplasma were recovered. All strains were digitonin positive, and 67 (95.71%) strains were biochemically glucose— (G-ve) and arginine—negative (Ar-ve) with production of film and spots (FS+ve). The remained strains (n = 6) gave variable biochemical results. To minimize
the cost of PCR technique, 50 (74.63%) strains of the G-ve, Ar-ve and FS+ve strains were randomly selected and PCR—
tested. All PCR—tested strains was Mycoplasma bovis

(Fig.1A&B) Lung sequestration with thickening and fibrosis of the interlobular septa (A). Cut section in the diaphragmatic lobe revealed a much of yellowish pussy material (B). N.B: the bluish coloration of the lobes

(Fig. 2) Cut surface of pneumatic lung-lobe, caseonecrotic bronchopneumonia with bronchiectasis and yellowish caseated purulent materials inside bronchioles. The cut surface was edematous, and purulent material was found in small airways. (Fig3): pulmonary sequestration with obviously demarcation of interlobular septa Fig

Discussion

BRD is a field problematic syndrome of cattle herds with negative economic impacts due to morbidity, mortality, treatment and prevention costs, loss of production and reduced carcass value. Environmental and management factors rather than pathogens play significant roles in the prevalence of BRD. Bad hygienic measures, accumulation of fecal matter and urine beneath animals with poor ventilation lead to increase ammonia level, which has, worsen effect on animal respiratory system and considered in a holistic approach to reduce BRD (Griffin, 1997 and Taylor et al., 2010).

The necropsy findings of the currently examined cattle indicated that the all-pulmonary lobes were bluish in color particularly the apical and cranial lobes. The diaphragmatic lobes were consolidated cranioventrally and cyanosed due to hypoxic hypoxia as necrosis to lung alveoli and consequently failure of blood-oxygenation with increased carboxyhemoglobin. Cut sections in the deepest portions of the affected lungs revealed a much amount of pussy and/or caseated material. This may refers to infection with pus-producing bacterial pathogens. Similar pathological caracterizations in calves’ lungs experimentally infected by Mycoplasma bovis was carried-out by Prysliak et al. (2011).

Currently, all culturally examined samples were

Table 1: Nucleotide sequences of the used PCR primers for detection of M. bovis:

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>16S rRNA</th>
<th>Sequences 5´ to 3´</th>
<th>Product size (base pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma bovis</td>
<td>MboF</td>
<td>CCT TTT AGA TTG GGA TAG CGG ATG</td>
<td>360 (González et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>MboR</td>
<td>CCG TCA AGG TAG CAT CAT TTC CTA</td>
<td></td>
</tr>
</tbody>
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(Fig.4): Characteristic fried-egg colonies of Mycoplasma on agar medium (x 32)

(Fig.5): Agarose gel electrophoresis of PCR amplification products of 16S rRNA gene of Mycoplasma bovis. Lane M: DNA Ladder (Marker) 100 pb. Lane 1: Control positive (Zaitoun, 2000). Lane 2: control negative. Lanes 3 10: positive tested samples with amplified products at 360 pb.
Mycoplasma positive and the majority of isolated Mycoplasma was glucose and arginine negative with production of film and spots. This may denotes to the occurrence of Mycoplasma bovis and/or Mycoplasma bovigenitalium, which are common pathogens of bovine respiratory system (Pruslyak et al., 2011 and Hashem et al., 2022). Presently, Mycoplasma bovis was molecularly detected in all PCR—tested samples referring to the significant role of this pathogen. Lung clearance mechanism and function of alveolar macrophages may impaired by Mycoplasma bovis, which facilitated the way of pus-producing bacterial pathogens and others to cause multiple pathological alterations including fibrosis, increase in thickness of interlobular septa, edema and fibrosis. Askar et al (2021) concluded that Mycoplasma bovis causes various chronic inflammatory diseases, including mastitis and bronchopneumonia, in dairy and feed cattle and suppresses the host immune response during infection, leading to the development of chronic conditions. This due the capability of Mycoplasma bovis produces proinflammatory cytokines and chemokines in the infected host that cause pathological alterations including inhibition of phagocytosis with immune damage (Askar et al., 2021). Moreover, Mycoplasma bovis modifies the functions of neutrophils of the infected animal to support its persistence and systemic dissemination and causes chronic bronchopneumonia with caseous pathological alterations and characterized by persistent infection that seems poorly responsive to many antibiotics (Caswell and Archambault, 2007 and Jimbo et al., 2017). This may interprets the failure of therapeutic trials attempted by field veterinarians and may refers to the chronological persistence of that infection. Consequently, the current work strongly signifies Mycoplasma bovis as a major pneumonic pathogen of BRD in Sohag Governorate and it is suggested that vaccination program against the most common pneumogens including Mycoplasma bovis should be regularly warranted.

Conflict of Interest:

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

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