



## Research Article

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**Clinical Study with Molecular Detection of *Mycoplasma Bovis* Pneumonitis in Imported African Breed Cattle in Abu Simbel, Egypt**Ahmed M.A. Zaitoun\*<sup>1</sup>, Doaa M.A. Salman<sup>2</sup>, & Ahmed M.E.Y. Kounour<sup>2</sup><sup>1</sup>Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt<sup>2</sup>Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Sohag University, Egypt**Article History**

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**Abstract:** From Mar. 2020 to Apr. 2022, a total number of 350 imported African breeds were clinically inspected for signs of respiratory troubles in Abu-Simbel Quarantine Station. One hundred twenty (34.28%) of the inspected cases showed outstanding signs of Bovine Respiratory Disease (BRD). Seventy-five cases of the inspected cases underwent emergency necropsies, and their lungs experienced culture testing for *Mycoplasma* infection. Grossly, the lungs demonstrated several regions of characteristic sequestrations in the investigated cases (66/75, 88%) with significant thickening and fibrosis of the interlobular septa. Furthermore, the tiny airways were clogged with caseated purulent exudate and had caseous necrosis bronchopneumonia. The culturally tested lungs were *Mycoplasma* were positive. According to the genus determination by digitonin test the eighty isolated strains were *Mycoplasma*. *Mycoplasma bovis* was molecularly positive using PCR with species-specific primers. The imported African breed's cattle may play a crucial role in the spread of *Mycoplasma* illness. The outcomes are extremely indicative. A vaccination program against *Mycoplasma bovis* is obligatory as it is a prominent pneumogenic agent of BRD.

**Keywords:** Molecular Detection, African Breed Cattle, Egypt, BRD.

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**INTRODUCTION**

Among the most common issues limiting the ability of cow herds to produce and reproduce is pneumonitis (Sasaki *et al.*, 2022). Severe pneumonitis has multiple factors. The main causes of severe pneumonitis in cattle herds are infections, stress-related factors, and poor management (Sayed & Zaitoun, 2009; Snowden *et al.*, 2009; Gaeta *et al.*, 2018; & Ammar *et al.*, 2022).

*Mycoplasma* infection is among the most prevalent respiratory pathogens in both large and small ruminants, according to a survey of the literature on the subject (Zaitoun, 2001; & Hashem *et al.*, 2022). However, *Mycoplasma bovis* has been repeatedly implicated as the main pathogen that causes respiratory disease in large ruminants (Nicholas, 2011; Mahmood *et al.*, 2017; & Ammar *et al.*, 2022).

It is yet unclear how *Mycoplasma* infection contributes to respiratory illness in Upper Egypt. Despite the bacteriological, immunological and epidemiological characterizations of calves' respiratory disorders in certain areas of the mid and Upper Egypt were elucidated by El-Seedy *et al.* (2020). Eight of the sixty respiratory-ill calves in Sadat City (Menoufiya Governorate, North of Egypt), according to Hashem *et al.* (2022), had *Mycoplasma* infections in their nasopharynxes. They concluded that *Mycoplasma* infection rates were higher than those of the bacterial pathogens. In the southern

governorates of Egypt, the current situation of *Mycoplasma* infection in imported African breed cattle with indications of BRD appears to be sparse. Therefore, the current investigation was attempted to identify the rate of *Mycoplasma* infection in cattle with severe pneumonias, specifically *Mycoplasma bovis*, in the lungs tissues of imported African breed cattle.

**MATERIAL AND METHODS****Ethics Approval**

The Faculty of Veterinary Medicine at Sohag University followed all experimental protocols that were approved by the committee's consideration of animal research.

**Animal**

From March 2020 to April 2022, a total number of 350 imported African breed cases were subsequently inspected clinically with various degrees of respiratory manifestations in Abu-Simbel Quarantine Station, Aswan Governorate, and Upper Egypt. One hundred twenty (34.28 %) of the inspected cases showed severe signs of BRD. Seventy-five cases were emergency necropsied due to the undesirable situations. Gross descriptions of their lungs were made, and their cultures were checked for *Mycoplasma* infection.

**Specimens and Laboratory Techniques**

Pneumonic lung tissue samples from the necropsied cases were aseptically removed and placed

right away in screw-capped bottles containing Mycoplasma broth culture supplemented with (bacterial inhibitors and growth promoters for bovine Mycoplasmas, as previously described by Zaitoun, 1990). 37°C was used for broth incubation. The incubated broths were routinely sub-cultured in fresh broths and incubated two days later. There were three blind passages completed. The incubated broths followed by plating onto Mycoplasma agar media and cultured in a gas-pack jar for two days in a 10% CO<sub>2</sub> atmosphere. Digitonin test was applied to differentiate between Mycoplasma and acholeplasma genera according to (Tully, 1983). A standard PCR approach was used to identify the purified mycoplasma colonies that were sensitive to digitonin.

Species-specific primers are used in PCR tests for Mycoplasma bovis as the most prevalent types. According to the directions supplied by the manufacturer of the QIAamp® DNA Mini Kit, DNA was extracted from the testing and control samples (Qiagen, Hilden, Germany, catalogue no.: 511304). The forward and reverse sequences of *Mycoplasma bovis* PCR primers were selected according to (González *et al.*, 1995); Forward: 5'-CCT TTT AGA TTG GGA TAG CGG ATG and reverse: 5'-CCG TCA AGG TAG CAT CAT TTC CTA T. The Biotechnology Unit of the Faculty of Veterinary Medicine, Sohag University, Egypt, followed the PCR method protocol for the analyzed samples based on Kounour (2018). A 50 µl reaction container was created and contained 25 µl of the HotStarTaq Master

Mix (Qiagen, Hilden, Germany, catalogue number: 203443), 1 µl (10 pmol/L) of each primer, 4 µl of DNA, and the remaining 50 µl of RNase-Free Water. A thermal cycler was used to conduct the PCR process (Sensoquest GmbH LabCycler, Göttingen, Germany). During PCR, the manufacturer of the kit's standard temperature profile was followed. Each primer pair for Mycoplasma bovis was annealed at a temperature of 60°C. The PCR results were examined using agarose gel electrophoresis (1.8% agarose gel) and documented using a gel documentation system.

## RESULTS

The necropsy examination revealed sequestrations in the pneumonic lungs of the investigated cases (66/75, 88%) with thickening and fibrosis of the interlobular septa (Fig.1). Moreover, the tiny airways were clogged with caseated purulent exudate and had bronchopneumonia with caseation and necrosis (Fig.2).

### The Tested Cases with Mycoplasma Infection

The culturally tested lung samples were Mycoplasma positive and 80 strains were recovered (Fig.3). All strains were digitonin sensitive. The fifty (62.5%) strains of Mycoplasma were randomly chosen and PCR-tested for *Mycoplasma bovis* in order to reduce the cost of the PCR procedure and due to *Mycoplasma bovis* is the most prevalent type. *Mycoplasma bovis* was found in every PCR-tested strain (Fig.4).

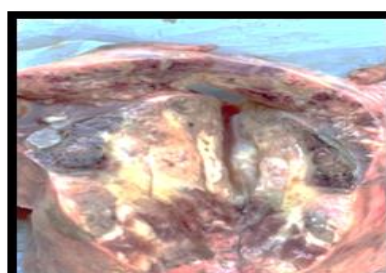
**Table 1.** Rate of infection with Mycoplasma positive cases of the animals

Animal	Nº. of the inspected cases	Nº. of the clinically suspected cases with signs of BRD	No. of the necropsied cases	Nº. of tested animals with mycoplasma positive cases
Imported African Breed cattle	350	120	75	75*

\*: All Mycoplasma tested samples were positive



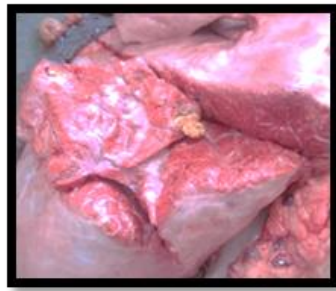
**Fig.1A**



**Fig.1B**

(Fig.1) The interlobular septa was filled with edematous fluid, fibrosed and thickened with pulmonary sequestration (A). Transverse section of pneumonic lung

of imported African breed bull emergency slaughtered with signs of severe pneumonitis inside slaughtered house with fibrin deposition and lung sequestration (B).



**Fig.2A**



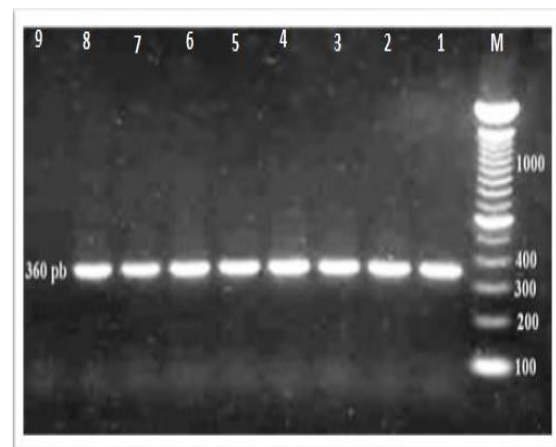
**Fig.2B**

(Fig. 2) Edematous surface of the incised lung lobe, bronchopneumonia with necrosis and caseation, and the tiny airways was filled with suppurative caseated

mass (A). Inside the pulmonary parenchyma, there was yellowish caseated purulent debris (B).



**Fig.3:** Mycoplasma colonies with recognizable fried-egg morphology on agar media (x 32)



**Fig.4:** 16S rRNA gene amplification products from *Mycoplasma bovis* were electrophoresed on an agarose gel.

DNA ladder of 100 bp, Lane M (Marker). Lane 1: (Zaitoun, 2000) control positive. Lane 9: control negative. Lanes 2-8: positive tested samples with amplified products at 360 bp.

## DISCUSSION

Due to morbidity, mortality, treatment and preventative expenses, loss of output, and decreased carcass value, respiratory disease is a field-problematic illness that affects cattle herds. Environmental and organizational variables, as opposed to infections, play a crucial role in the development of pneumonia. Poor hygiene practices, long transportation distances, stocking densities with poor ventilation, and commingling all enhance the effects on animal respiratory systems and must be taken into account in a comprehensive strategy to eliminate respiratory disease in cattle (Griffin, 1997; & Snowden *et al.*, 2006).

Rendering to the necropsy results of the investigated cattle revealed that the all-lung lobes were cyanosed. Failure of blood oxygenation due to lung

alveolar necrosis and the caudal lobes were consolidated. The type of bronchopneumonia that was most common was caseonecrotic. Transverse sections in the parenchyma of pneumonic lungs showed a much amount of suppurative and/or yellowish caseated masses. This might be a suppurative bacterial infection. Hermeyer *et al.* (2012) described similar pathogenic characteristics in *Mycoplasma bovis*-infected calves' lungs.

During this investigation, all culturally studied samples tested *Mycoplasma* positive, and were sensitive to digitonin. *Mycoplasma bovis*, which is the frequent pathogen of the bovine respiratory system, is mentioned as possibilities in the literature (Hamad *et al.*, 2019; & Valeris-Chacin *et al.*, 2022). All PCR-tested samples included *Mycoplasma bovis*, which highlights the crucial role that this pathogen plays. *Mycoplasma bovis*, which facilitated the passage of purulent bacterial pathogens and others to produce many pathological modifications including fibrosis, increase in thickness of interlobular septa, edema, and fibrosis, may disrupt the lung clearance mechanism and function of alveolar macrophages. Maunsell *et al.* (2011) asserted that

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*Mycoplasma bovis* suppresses the host immune response during infection, resulting in the development of chronic illnesses. This is due to the capability of *Mycoplasma bovis* produces chemokines and cytokines in the infected host that cause pathological alterations including inhibition of phagocytosis with immune damage (Maunsell & Chase, 2019). Additionally, *Mycoplasma bovis* causes chronic bronchopneumonia with caseous pathological alterations and is characterized by persistent infection that appears poorly responsive to many antibiotics (Caswell & Archambault, 2007; & Bürki *et al.*, 2015). It also modifies the functions of neutrophils of the infected animal to support its persistence and systemic dissemination (Gondaira *et al.*, 2021).

Furthermore, from an epidemiological perspective, the data show that the prevalence of mycoplasma infection in Imported African breeds (75/99) was 75.75%. This could be crucial in the spread of the infection in different parts of Egypt with different field issues (Zaitoun, 1990; Zaitoun *et al.*, 1991; Zaitoun, 2000; & Hashem *et al.*, 2022). Therefore, it is concluded that vaccination programs against the most frequent pneumogens, including *Mycoplasma bovis*, should be considered. The current investigation strongly indicates that *Mycoplasma bovis* is a prominent pneumogenic pathogen causing respiratory illness in cattle. Further research should be done to determine the function of respiratory viruses and pus-producing pathogens in respiratory diseases in cattle.

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