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Detection of *Trueperella pygogenes* in deep pulmonary tissues of cattle with suppurative pneumonia in Sohag governorate, Egypt

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ABSTRACT

Within three years investigation (2020 – 2022), 50 cattle in Sohag governorate, South Egypt, were necropsied due to severe respiratory manifestations. Their lungs were grossly and histopathologically monitored, and culturally examined for detection of pus-forming pathogens. All examined cattle were unvaccinated against respiratory viral pathogens. Lung-sequestrations with whitish-yellow purulent and/or caseated masses with necrosis of deep tissues was the topmost necropsy finding. The lung appeared as an edematous structure containing yellowish caseated purulent materials with demarcation of the interlobular septae. Histopathologically, the interlobular septae were filled with fibrinous exudate with fibrinopurulent inflammation in alveolar tissue. Bacteriologically, *Trueperella pyogenes* followed by *Staphylococcus aureus*, *β hemolytic streptococci* and non-hemolytic short chain cocci were culturally isolated with frequency percentage of 100%, 42.00%, 18.00% and 18.00%, respectively. The highest frequent isolation of *Trueperella pyogenes* from the deep pulmonary tissues may symbolize the existence of an enabling factor facilitate the pathway to deep pulmonary tissues by damaging the lung defense mechanism. Similarly, *Staphylococcus aureus*, β hemolytic streptococci, and non-hemolytic short chain cocci are also pus-forming pathogens and they follow the same path of *Trueperella pyogenes*. The existence of pyogenic pathogens increased the seriousness of pneumonias in cattle and declined the chances of successful therapy. The prime enabling agents that facilitate the pathway of pyogenic microbes to invade the deep respiratory system should be screened. Cattle should be regularly vaccinated against the respiratory pathogens deterring the lung clearance mechanism to reduce the existence of pyogenic pathogens. Investigation on role of respiratory viruses and mycoplasmas in pneumonias are crucially reasonable.

1. INTRODUCTION

Pneumonias are a serious field problem of cattle significantly deters the level of productive and reproductive capacities of the herd and/or individual cases (Griffin et al., 1997 and Decaris et al., 2022). Bovine pneumonias are a multifactorial syndrome. Stress factors, bad management in association with various pathogens appear to be the major factors triggering Pneumonias (Gaeta et al., 2018 and El-Seedy et al., 2020)

Etiologically, there are several pathogenic agents were implicated as pneumonic pathogens encountered by Quinn et al (1994) and Murray et al (2017) Bacterial pathogens may play a noteworthy role in bovine pneumonias (Zecchinon et al., 2005). The defense mechanisms of infected cattle and other ruminants are suppressed by *Mannheimia hemolytica* and its leukotoxin, they emphasized, creating an environment that is favorable for the invasion of additional infections. On the other side, Yates (1982) and Lopez (2001) found that respiratory viruses particularly bovine herpesviruses were more prominent pathogens than bacteria in induction of bovine pneumonias. They also declared that the respiratory viruses damage the windpipe allowing bacteria enter the deep respiratory system of the infected cattle. The viral-bacterial synergism in bovine pneumonias was previously accounted by Fulton (2009)

Likewise, Mycoplasma infection is plays a pivotal role in bovine and ovine respiratory disease and is recurrently incriminated as an outstanding primary pathogen responsible for seriousness of pneumonias during the last years in Egypt (Zaitoun, 2001 and Hashem et al., 2022)

In Menoufiya Governorate, Egypt, Hashem et al. (2022) found that *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Pasteurella multocida*, and *Staphylococcus aureus* were present in 8.33%, 5%, 5%, and 5% of the tested nasopharyngeal swabs, respectively, and were frequently linked to respiratory symptoms in young calves. Zaher et al. (2014) detected *bovine herpes virus-1* (7.49%), *parainfluenza virus-3 (0.44%)*, bovine *respiratory syncytial virus* (0.44%) and *Bovine viral diarrhea* (5.29%) using Dot ELISA, PCR and electron microscopy, and *Mannheimia haemolytica* (3.08%) and *Pasteurella multocida* (2.20%) by culturing techniques onto 5% sheep blood and MacConkey's agar in mature buffalo cows with respiratory

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manifestations at Al Sharquia and Al Daqhaliya Governorates in Egypt

There are different diagnostic techniques used in the diagnosis of the causative agents of bovine respiratory disease, including microbial culture, polymerase chain reaction, serology (antibody enzyme-linked immunosorbent assay), and nanosequencing. However, microbial culture is still the gold standard technique (Pardon and Buczinski, 2020)

Briefly, *Trueperella pyogenes* is a gram-positive coccobacillus that acts as a ubiquitous opportunist capable of establishing chronic pyogenic infections virtually anywhere in the cow's body including suppurative pneumonia (Rzewuska et al., 2019). Previously, *Trueperella pyogenes* was formerly named *Arcanobacterium* (*Actinomyces*) *pyogenes* that initially described by Glage in 1903. Prior to being moved to the genus Actinomyces in 1982, it was known as *Corynebacterium pyogenes*. It has been referred to as *Arcanobacterium pyogenes* since 1997 (Ramos et al., 1997). The new genus was named in honour of German microbiologist Hans G. Trüper when the genus Arcanobacterium was divided into two (Arcanobacterium and Trueperella). The term "pyogenes," which is used to describe a variety of bacterial genera, comes from the Greek or Latin words "puon" or "pyum," respectively, and the suffix "-genes, producing pyogenes," which means "pus-producing" (Yassin et al., 2011)

Trueperella pyogenes was isolated in Egypt from the pneumonic lungs of camels slaughtered in Cairo Governorate with 1.4% (Wareth et al. 2014), the uterine discharge of dogs and cats present in a dairy cattle farm with a history of frequent episodes of mastitis and abortion (Wareth et al. 2018), and the mastitic milk of dairy buffaloes in different areas of Sohag Governorate with 2.5% (Zaitoun et al. 2019)

During the past three years (2020 – 2022), subsequently several cases of cattle succumbed or emergency slaughtered were reported in different villages of Sohag Governorate, South Egypt, due to unsuccessful therapeutic trails with progressive worsen respiratory conditions. They were necropsied and their lungs were grossly suppurative. Consequently, exploration of pus-creating pathogens in pneumonic lungs of necropsied cattle was aimed in the current work

2. MATERIAL AND METHODS

2.1. Samples and sampling procedures

Within three consecutive years (2020 – 2022), fifty swabs of severely pneumonic lungs of necropsied cattle died due to bad-sequel of acute pneumonia were subsequently taken particularly from the pussy and/or caseated materials. The collected samples of pneumonic lungs were kept in an ice box and transported immediately to the laboratory of Infectious Diseases in the Faculty of Veterinary Medicine at Sohag University with minimum delay. In the laboratory, the samples are bacteriologically cultured immediately. After that, stored in a deep freeze - 20ºC

2.2. Cultural and biochemical identifications of pus-forming bacteria

Detection of pus-forming bacteria was carried out based on the protocol illustrated by Quinn et al. (1994). Briefly, the swabs were immediately immersed in screw-capped bottle containing brain-heart infusion broth (BD) supplemented with 5% commercial heated-inactivated horse serum (Merck®, Darmstadt, Germany, catalog no.: H1138 Sigma-Aldrich). The broth tube was incubated at 37ºC for 24 – 48 hours and thereafter platted onto the following media, 5% sheep blood agar, Egg-yolk-potassium tellurite-Baird Parker agar and *Streptococcus* selective agar (BD). The cultured plates were incubated aerobically for 24 – 48 hours with exception of SSA's plats incubated in low-oxygen tension environment (candle Jar incubation). Post incubation, the suspected colonies were picked-up and subculturally purified and thereafter stained by Gram stain. The Gram-positive purified stained bacteria were identified morphologically and biochemically based on the criteria of Quinn et al (1994). These criteria include colonies characteristics, hemolysis on blood agar, Gram staining, growth on mannitol salt agar and MacConkey's agar media, catalase, oxidase, wet-mount-motility, Rabbit's plasma coagulase test, and CAMP and reserve CAMP reactions. On the other hand, the Gram-negative colonies were slanted on BHI-agar slops and stored in refrigerator.

2.3. Histopathology

Small tissue specimens from the affected parts of the pneumonic lung were excised, trimmed, fixed in 10% buffered neutral formalin and then embedded in paraffin. Five µ thick paraffin sections were routinely prepared and stained with hematoxylin and eosin (Suvarna et al., 2013). Thereafter, they examined microscopically.

3. RESULTS

Grossly, the foremost necropsy findings of the necropsied cattle were lung-sequestrations with whitish yellow purulent caseated masses and necrosis in the deep lung tissues (Fig. 1). Furthermore, a lung abscess was obviously noticed, with marbling on the outside and enlarged, edematous, and demarcation of the interlobular septae (Fig. 2)

The histopathological examinations of the examined lungs elucidated that the interlobular septa were remarkably filled with fibrinous exudate in association with fibrino-purulent inflammation in alveolar tissue (Fig.3). The lung alveoli were obliterated by suppurative exudate with thickening of alveolar wall with hyperplasia of pneumocytes type II (Fig 4)

The bacteriological examinations of the examined cases were summarized and illustrated in Table 1, which obviously indicated that *Trueperella pyogenes* is a more predominant pathogen rather than others pathogen; *Staphylococcus aureus*, β hemolytic streptococci and non-hemolytic short chain cocci where the percentage isolation of these pathogens were 100%, 42.00%, 18.00% and 18.00%, respectively (Table 1)

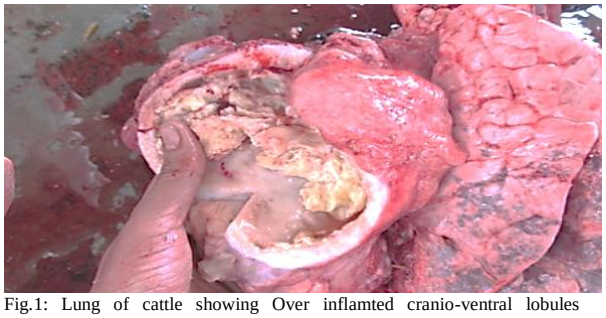

Fig.1: Lung of cattle showing Over inflamted cranio-ventral lobules interspersed with darker pneumonic lobules with whitish yellow purulent masses and necrosis of deep lung tissues and pulmonary sequestration

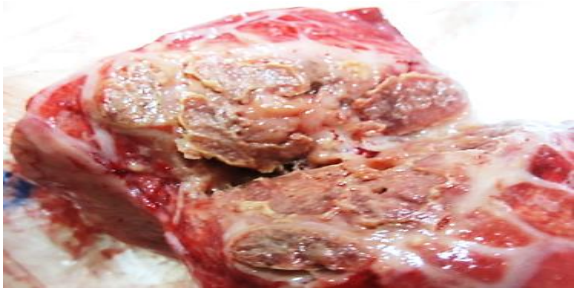

Fig.2: Lung abscess (Lung appear as bag contain brownish caseated purulent material) with marbling external appearance and well demarcated interlobular septae

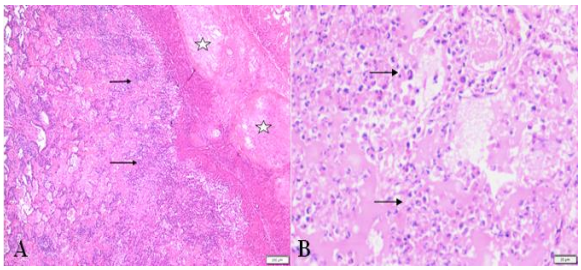

Fig.3: Photomicrographs of lung tissue infected with pus-forming pathogen showing (A (40x) magnified in B (400x)): fibrino-purulent inflammation in alveolar tissue with massive neutrophil cell infiltration (arrows), interlobular septa filled with fibrinous exudate (stars). H&E stain

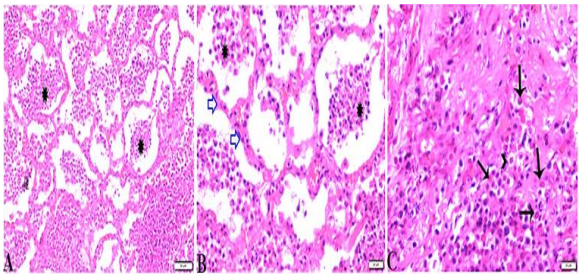

Figure 4: Photomicrographs of lung tissue infected with pus forming pathogen showing (A (200x) magnified in B(400x)): lung alveoli oblitrated by suppurative exudate (stars). Thikining of alveolar wall with hyperplasia of type II pneumocytes (white arrows). (C): marked inflammatory cellular infiltration (arrows), hyaline droplets (arrowhead) (400x). H&E stain A

Table (1): Pus-forming pathogens isolated from lungs of the necropsied cattle

Pathogen	No. of cases (n = 50)*	Percentage
Trueperella pyogenes (single infection)	29	58%
Trueperella pyogenes (mixed infection) *Staphylococcus aureus*	12	24%
Trueperella pyogenes (mixed infection) β hemolytic streptococci *Staphylococcus aureus* Non-hemolytic short chain cocci	9	18%

The percentages isolation of *Trueperella pyogenes*, *Staphylococcus aureus*, B hemolytic streptococci *and* non-hemolytic short chain cocci were 100%, 42.00%, 18.00% and 18.00%, respectively

4. DISCUSSION

Bovine pneumonia considers a major enemy for dairy and/or beef cattle breeding and remains as reviewed by (Panciera and Confer, 2010).They elucidated that pneumonia remains a most important cause of illness and death with a considerable level of financial losses. When cattle are exposed to stress factors, like shipment, and commingling with animals from other sources, transmission of various respiratory pathogens and propagation of endogenous yet potentially infectious agent occur resulting in deterring the defense mechanism of the respiratory system with subsequent upper or lower respiratory illness (Andrews and Kennedy, 1997). Unfortunately, the cattle population, now, in Sohag Governorates, contains a huge number of African breed cattle with diminution of the baladi breeds particularly in beef farms. This may induce a favorable chance for existence of new strains of respiratory pathogens and others microbial agents

Currently, the existence of pussy and/or caseated materials in the deep parenchymatous structure of lungs with characteristic histopathological findings, which indicated that there were interlobular septa remarkably filled with fibrinous exudate in association with fibrinopurulent inflammation in alveolar tissue of the examined cases may denote to invasion of pus-creating pathogens. The bacteriological examinations revealed *Trueperella pyogenes* followed *Staphylococcus aureus*, β hemolytic streptococci and non-hemolytic short chain cocci. These isolates are suppurative bacteria (Quinn et al., 1994). The frequent percentages of the isolated pyogenic pathogens, *Trueperella pyogenes*, *Staphylococcus aureus*, β hemolytic streptococci and non-hemolytic short chain cocci from the bacteriologically examined cases were 100%, 42.00%, 18.00% and 18.00%, respectively. The bacteriological results offered by Zhou et al (2023) concluded that the *Trueperella pyogenes* (9.37%) was more prominent pneumogenic agent than *Pasteurella multocida* (8.35%), *Mannheimia hemolytica* (2.44%), and other bacteria (7.13%). This may refer to a significant role of *Trueperella pyogenes* in increases the seriousness of cattle's pneumonia

Additionally, a wide range of pyogenic clinical symptoms in animals have been linked to Trueperella pyogenes as their principal causative agent (Ribeiro *et al*. 2015 and Rzewuska et al. 2016). It is an organism ubiquitous in nature and considered one of the ordinary inhabitants of the mucous membranes of upper respiratory system, urinary tract, genital tract, and skin of the animals (Rzewuska et al., 2019). Consequently, the existence of the isolated pathogen in parenchymatous pulmonary tissues (diaphragmatic lobes) of the examined animals may ascribed to the presence of enabling agents that hinder the lung-defense mechanism and subsequently seriousness respiratory illness are established. Respiratory virus and mycoplasma are suggestive. Moreover, the existence of suppurative material due to pus-creating pathogens in lung of cattle may hamper the efficient regime of therapeutic trails with newest antimicrobial drugs.

5. CONCLUSION

Finally, it is concluded that the prime enabling agents that facilitate the pathway of pus-forming microbes to invade the deep respiratory system should primarily screened. Cattle should be regularly vaccinated against the respiratory pathogens deterring the lung clearance mechanism to minimize the risk of infection and to reduce the invasion of the existed pus-forming pathogens. Investigation on role of respiratory viruses and mycoplasmas in bovine pneumonias are reasonable to clear-up the common pneumogens.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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