

## **A case study of pneumonic changes and death in Nilgai (*Boselaphus tragocamelus*) with respiratory disease in Punjab, Pakistan**

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### **Abstract:**

A nine-year-old Nilgai (*Boselaphus tragocamelus*) in Bahawalpur Zoo, Punjab, Pakistan, was ill with respiratory disease, so the pathogens involved were studied, and morphological features and propensity for Bovine Respiratory Disease (BRD) involved were also speculated. Because of demolition, Nilgai is sparsely populated in Pakistan, so studying the cause of its death was essential. The lung samples were collected, and histopathological techniques and bacterium isolation were used to analyse them. Necropsy, i.e., gross and histopathological postmortem exams, were carried out in the laboratory using customary methods under rigorous crafting circumstances. A standard visual and microscopic methodology was used in gross examination, and routine microbiologic tests were run. Specific and compelling infectious agents have been found in Nilgai's lungs, including *Pasteurella multocida* and *Mycoplasma bovis*. Pathology and histopathology revealed that Nilgai had extensive ulceration and chronic swelling behind his mandibles, and the post-mortem report verified this. The report revealed that the lungs were grey hepatized. Histological lesions represented acute, severe, haemorrhagic, and chronic bronchopneumonia stages of pneumonia. Lungs with histological lesions were classified as pattern pneumonia. The current study's goals were to report clinical, pathologic, and molecular data and to determine the cause of the respiratory ailment that affected one Nilgai.

**Keywords:** Nilgai, hepatization, lesions, necropsy, pathogens, pneumonia, ulceration, respiratory disease, bovine respiratory disease.

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## 1. Introduction

Nilgai or Blue cow (*Boselaphus tragocamelus*) is the Asia's largest antelope (Leslie & Schaller, 2008). It is the only species of the genus *Boselaphus*, which Peter Simon Pallas initially described in 1766. Interleukin 2 and prion protein gene sequences indicate that Nilgai and water buffalo *Bubalus bubalis* share a common ancestor (Dhara *et al.*, 2007). Adult males are bluish-grey, while females and juveniles are orange to blonde. It has been imported to Italy, Mexico, United States (Texas), South Africa and is native to promontory India and minor portions of Nepal and Pakistan. It is a major herbivorous mammal in India, favouring open grassland and savannas (Rafferty, 2010). It is present in large numbers in zoological gardens and privatized collections worldwide and is not particularly threatened by conservation efforts (Schmidly & Bradley, 2016).

Nilgai is diurnal (active primarily throughout the day). An investigation into the antelope's daily behaviour revealed feeding peaks at dawn, in the morning, in the afternoon, and in the evening (Oguya & Eltringham, 1991). Females and juveniles do not engage with males much except during mating season. The typically docile Nilgai may appear timid and cautious when agitated or alarmed. Rather than running for cover like duikers, they will instead run up to 300 m or even 700 m away from the threat while galloping (Sheffield, 1983). The Nilgai are not commonly affected by acute or sub-acute illnesses, and epidemics are uncommon. However, bovid lung and liver cancer are prevalent in Nilgai (Dudley & Willett, 1965). Nilgai populations in India that are free to roam likely contact illnesses from domestic animals (Singh *et al.*, 2010). Respiratory illnesses are the primary killers of Nilgai (Gallagher *et al.*, 1998), and these respiratory illnesses are regarded as Bovine Respiratory Diseases (BRD) in cattle's. BRD is a multi-factorial syndrome, and the participation of certain components has been challenging to determine due to its intricacy.

Lung lesions, a single round or oval growth in the lung, referred to as a lung strain or Solitary Pulmonary Nodule (SPN), primarily brought on by pneumonia and tuberculosis, are the causes of respiratory illnesses (Paśnik *et al.*, 2017). The lesions come in a variety of forms. Emphysema, congestion, hydatidosis, and atelectasis have been identified as the most significant lesions (Zeryehun *et al.*, 2017). The lung lesion may be brought on by lung cancer, carbuncle, ulcer, or wound (Johnson & Pendell, 2017). A biopsy may be carried out to determine if the nodule or lesion is malignant. Pneumonia, a bacterial or viral infection that results in pus-filled air sacs that may solidify, is another key factor in the inflammation of the lungs and death of cattle's (Madsen *et al.*, 2017).

Tuberculosis (TB) is the other common kind of respiratory disease. Blood vessels can be damaged by the developing sickness, which causes the diseased animal to cough up brilliant red blood (Earley *et al.*, 2017). The condition is characterized by the development of hard nodules/lesions or big cheese-like lumps (Ranjan *et al.*, 2018). Bacterial microbes such as *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, and *Mycoplasma bovis* are the main disease causing organisms (Abdeen *et al.*, 2017). In the cattle sector, the BRD is a welfare and financial concern. Due to the mortality, treatment expenses, and difficult to calculate long-term expenditures, it causes significant economic short-term losses (Holman *et al.*, 2017).

Considering the above review, the study is designed to detect pathological carbuncles/lesions,

associated microbes and evaluate the consequences of BRD as a major source of mortality in adult bovid's posited for scrutiny.

## 2. Literature review

Buczinski and Pardon (2020) said the term “bovine respiratory disease complex” refers to bacterial bronchopneumonia that may or may not be complicated by prior or concurrent viral or bacterial infection. Numerous bacterial species can be identified from infected animals' lungs. Mannheimia (*Pasteurella*) haemolytica is the most important pathogen in feedlot cattle and adult cattle with *Pasteurella multocida* and *Hemophilus somnus* playing minor roles. These same infections are present in younger calves, but *Mycoplasma* spp. is also considered important. Although *Arcanobacterium pyogenes*, *Fusobacterium* spp., and *Bacteroides* spp. are often isolated from animals with chronic, abscessing lung lesions, they play no significant role in acute bronchopneumonia. Less common bacterial isolates, such as *Streptococcus* spp., and *Chlamydia* spp. are also seen in young calves.

Ollivett and Buczinski (2016) studied physiology of *Mycoplasma bovis*. Cattle respiratory illness is all brought on by the significant but frequently disregarded bacterium known as *Mycoplasma bovis*. It is widespread worldwide and has recently started appearing in new places, such as Ireland and some regions of South America. It causes at least 25 to 30 percent of all cases of calf pneumonia in Europe, though this number may be understated because, so few laboratories routinely check for mycoplasmas. Since *Mycoplasma bovis* lacks a cell wall, it is naturally resistant to some classes of antibiotics, like tetracycline, tilmicosin, and spectinomycin, which have been used to treat it in the past. Evidence is also mounting that some strains of *Mycoplasma bovis* are becoming resistant to these antibiotics. There are currently no vaccinations available to prevent *Mycoplasma bovis* infections.

Munir (2014) performed a prevalence study on Nilgai butchered in the community Abattoir of Hwassa to evaluate the prevalence of significant gross lung abnormalities. Routine inspection techniques were used to check the lung lesions after death. Calves that were evaluated had varied gross lung abnormalities after postmortem assessment. Emphysema, hydatidosis, congestion and atelectasis were the most significant lesions found. Incidence of body condition of slaughtered cattle and atelectasis were found to be statistically significantly correlated. Thus, the most common lung lesion in cattle slaughtered in the Municipal Abattoir of Hawassa was hydatidosis.

Verma *et al.* (2012) studied generalized tuberculosis in a Nilgai (*Boselaphus tragocamelus*), which was detected by *Mycoplasma fortuitum* isolation. The lung lesions could be seen as grossly as milliary lumps of varying sizes that were entrenched in parenchyma and somewhat uplifted from the surface and contained sleazy substance. Lung histopathological lesions are characterised by widespread caseous necrosis with occasionally calcified regions. A cellular response made up of lymphocytes, macrophages, and epithelioid cells encircled the necrotic patches. Except for a few locations where fusion-in-progress macrophages could be seen, giant cells were non-existent. The resulting minute granulomas were moved into the adjacent parenchyma and were not encircled by fibrous connective tissue.

Taylor *et al.* (2010) said the most expensive disease affecting beef cattle in North America is BRD. Cattle are predisposed to pneumonia for several different physical and physiological

reasons, making it a multifactorial condition. However, determining which factors are most crucial has frequently fallen short of yielding conclusive results. The most dangerous time for cattle's is right after shipment and when they are in contact with domestic animals. It is not apparent if these behaviours represent subpar management, increased exposure or enhanced susceptibility. It is uncertain whether persistent infection calves influence other cattle in the feedlot, which makes it challenging to determine how persistent infection with the bovine virus affects other cattle in the feedlot.

Caswell *et al.* (2010) studied the effects of *Mycoplasma bovis*. According to the researcher's chronic bronchopneumonia, arthritis, and tenosynovitis in beef cattle have all been linked to *Mycoplasma bovis* recently. Affirmation suggests that *Mycoplasma bovis* colonizes lung lesions that were first brought on by different bacteria, including *Mannheimia haemolytica* and maintain them. Although *Mycoplasma bovis* can operate as a main pathogen, many instances also involve other bacteria or viruses. *Mycoplasma bovis* causes a strong humeral immune response, but because of the varied surface proteins, the resulting antibodies are not protective, and the vaccines have not yet been proved to prevent illness. The well-being of these animals is a crucial component of managing the health of feedlots since the *Mycoplasma bovis* infections are the primary cause of a significant amount of chronic disease that develops in the feedlots.

### **3. Material and methods**

#### **3.1. Case history and clinical observation**

A 9-year-old male blue bull was suffering from chronic swelling behind the mandibles. The animal was treated many times over last 3 years, but the swelling subsides over the time, and upon cessation of therapy, it starts reappearing and increasing. A week before his death, the swelling response towards therapy was very poor. It resulted in serious illness of the animal for which the animal has been given injectables and infusion therapies, but it could not recover and expired.

#### **3.2. Necropsy**

At the time of necropsy, it was discovered that the lungs had shrivelled and mended ulcers as well as extensive gumboils on the tongue's borders. Specially swollen and bleeding lymph nodes were seen in the lungs. Additionally, the mandible lymph nodes were significantly engorged and hyperemic. Abdominal mucosa displayed extensive ulcerations and was extremely congested. On the gastric mucosa's surface, some ulcers had hyperemic elevated margins and amber necrotic regions in the centre. A bile-filled gall bladder and lesion-filled lungs were discovered. Hemosiderin buildup was also seen in drained regions of the spleen due to the significant congestion and haemorrhages.

#### **3.3. Tissue sample collection histopathology**

Aseptically taken lung samples from butchered Nilgai were brought to the pathology laboratory of Quaid-E-Azam Medical College (QMC) Bahawalpur for examinations, including gross and histological abnormalities. The tissues were embedded in paraffin wax and then cut using a microtome to get thin sections. Rotating microtomes were used to slice sections that were 3 to

4 m thick. For histological analyses, the tissues were then stored in the neutral buffered formalin.

### 3.4. Gross and histopathology of lung and liver tissues

Lung tissue samples were obtained, and macroscopic observations, such as histological lesions, were noted. The size of all the pneumonic regions was recorded using a sketch. At autopsy, representative pneumonic lesions were removed from the calf and put in 10% concentration of neutral buffered formalin for histological analysis.

Table-1: Composition of neutral buffered formalin

Sodium hydrogen phosphate	6.5 g
Sodium dihydrogen phosphate	4.0 g
Formalin 37% (w/v)	100 ml
Distilled water	900 ml

### 3.5. Histopathological investigations

Tissue samples were processed for histopathology, as shown in Table-2 after being fixed for 24 hours in Bouin's solution, followed by 70% ethanol.

Table-2: Histological procedure for tissue processing

Protocol	Chemicals	Time (Hours)
Washing	Running water	10
Dehydration	Alcohol 70%	6
	Alcohol 85%	6
	Alcohol 95%	4
	Absolute Alcohol-1	2
	Absolute Alcohol-2	1
Clearing	Alcohol: xylene 1:1	½
	Xylene-1	½
	Xylene-2	½
Infiltration	Paraffin-1	1
	Paraffin-2	1
	Paraffin-3	1
Embedding	Paraffin wax	Immediately

These sections were subsequently deparaffinized, hydrated, stained with acid haematoxylin and eosin, and saved at 50 °C for 4 hours (Bancroft & Gamble, 2008). The tabular summary of the staining process is presented in Table-3.

### 3.6. Procedure for staining tissue sections

One of the most crucial elements in making a precise pathologic diagnosis is tissue staining. If staining is carried out incorrectly, it may be challenging to visualize and distinguish microscopic details. Sections of paraffin wax were created and stained with phloxine tartrazine, azure eosinate, haematoxylin and eosin, giemsa and Verhoeff-Van Gieson method for elastin and collagen (The strong staining of elastic fibres caused by VVG staining happens quickly.

The VVG stain comprises the van Gieson component, a counterstain specific for collagen, and the Verhoeff stain, an iron-hematoxylin stain specific for elastin fibres. For reticulin, Gordon and Sweet's method (which is a procedure of silver impregnation that shows reticular fibres. Reticulum, a component of the body's support system, is prevalent in the liver, spleen, and kidney) and Lendrum's method for inclusion bodies (This technique can be used as a replacement for the HPS if the differentiation in the tartrazine solution is sped up to preserve pink cytoplasm and muscle. It is also used to demonstrate Paneth cell granules) Lendrum *et al.* (1962).

Table-3: Staining procedure for tissue sections

Protocol	Chemicals	Time (Minutes)
Dehydration	Xylene-1	2
	Xylene-2	2
	Absolute Alcohol-1	2.5
Clearing	Absolute Alcohol-2	2.5
	Alcohol-70%	3
Washing	Running water	4
Staining	Hematoxylin	15
Dehydration	Acid alcohol	3 dips
Washing	Running water	4
Dehydration	Ammonia alcohol	2.5
Washing	Running water	4
Dehydration	Alcohol 70%	4
	Eosin Y	2
	Absolute Alcohol-1	2.5
Staining	Absolute Alcohol-2	2.5
	Xylene-1	2
	Xylene-2	2

### 3.7. Lendrum's method

Rehydrate distilled water after deparaffinization, one minute of acidified Lillie-Mayer or Harris hematoxylin, rinse three times with distilled water, 15 seconds of blue hematoxylin in 0.3% sodium borate, rinse with distilled water four times, 30 minutes of phloxine solution, drain slides thoroughly and rinse with three changes of distilled water. Wipe the tissue's surrounding area with extra water. The section will not differentiate consistently if this is not done. In tartrazine solution, differentiate until inclusion bodies stand out as brilliant red spots on a yellow background, dehydrate three times each in absolute alcohol and 95% alcohol, clear in three to four xylene changes, and mount using artificial resin.

## 4. Results

There was a gap of two days between the death and postmortem of Nilgai. During the postmortem examination, the lungs were examined for the presence of cysts or parasites, as well as other gross abnormalities, using visualisation, palpation, and incisions as needed. The postmortem reports concluded that Nilgai had pneumonia, characterized by congested lungs and lung consolidation. Additionally, the lungs were much heavier than usual. There are several stages to the pathological lesions of the lungs, including congestion, consolidation, grey

hepatization and resolution (Figure 1(a)). Nilgai experienced grey hepatization, in which the lungs are still solid but lose some of their red colour due to the loss of erythrocytes. Lung firmness is essentially unchanged. The trachea and bronchi had a lot of white, frothy fluid (Figure 1(b)). However, the causal agents were more deadly, leading to the animal's death from respiratory failure as well as long-lasting lesions like scar development. *Mycobacterium bovis*, and *Pasteurella multocida* are the main culprits (Figure 2 and Figure 3).

Although both of these attack at the lower respiratory tract, however the environmental and host factors which may be involved in the activation of these bacterial pathogens are discussed in Table-4. Various gross lesions of emphysematous types (granulomatous nodules) were discovered in Nilgai's lungs during the postmortem examination. Emphysema in the lungs, which led to chronic obstructive pulmonary disease and damage to the air sacs (alveoli) in the lungs, was brought on by the lesions. It resulted in an abnormally large and ongoing buildup of air in the lungs. As a result, body does not receive the necessary amount of oxygen. It is also known as fog fever or bovine pulmonary emphysema. Interstitial, alveolar, and secondary conditions of emphysema were seen. The Nilgai was emphysematous, despondent, slow to sleep, and had nasal discharge. Nearly half of the alveolar gaps and interlobular compartments were filled with gas bubbles. However, Lendrum's technique lung staining did not reveal any phloxinophilic inclusion bodies. These were Nilgai's primary causes of death. Similar clinical symptoms were seen in other calf's infections with *Pasteurella* and *M.bovis* bacterial pathogens as in this case.

Table-4: Factors involved in BRD

Bacteria	Environmental Factors	Host Factors
<i>Mycoplasma bovis</i>	Freezing weather, dust, inadequate oxygenation and ventilation	Persistent infection with BVH-1, diarrhea, castration, less immunity.
<i>Pasteurella multocida</i>	shipping	High risk in male cattle's

Viral and bacterial infections interact to cause BRD, a multi-factorial illness. Stress (physical or physiological) brought on by unfavourable environmental factors such as exceptionally bad weather, ineffective management, overcrowding, and transportation can cause it.

Figure 1: (a) Grey hepatization, and (b) Frothy material

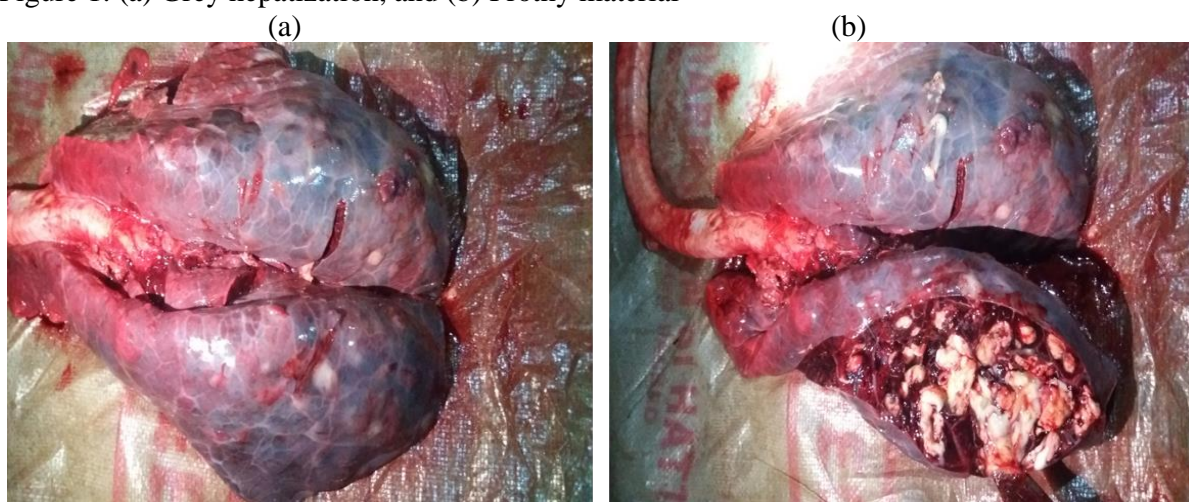
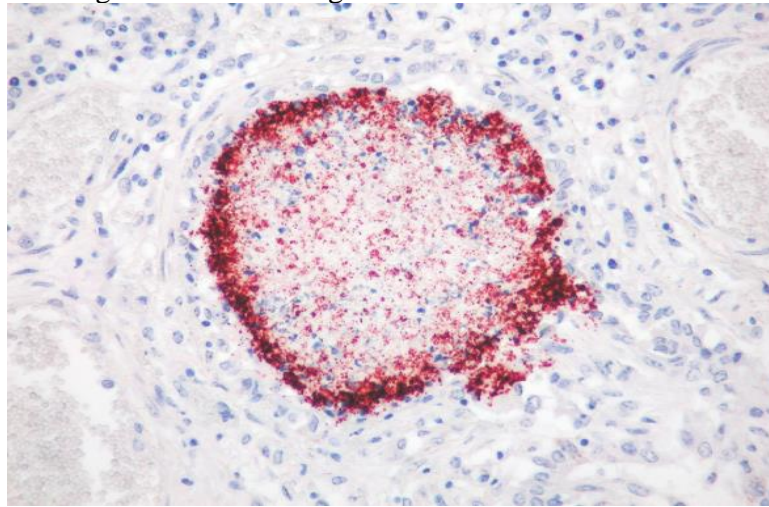
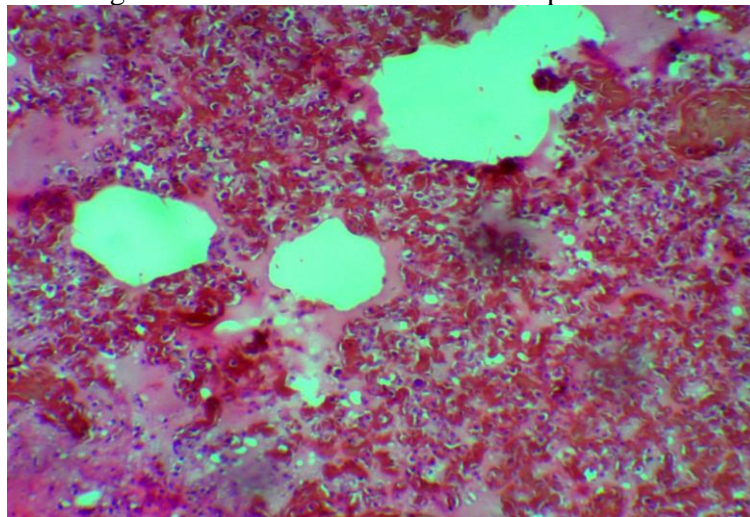




Figure 2: Red staining of *M.bovis* around bronchioleFigure 3: *P. multocida* acute bronchopneumonia

## 5. Discussion

The primary purpose of this analysis was to fill the knowledge gaps regarding the pathogens, pneumonia patterns, and related epidemiologic risk factors of BRD in Nilgai, which was submitted for postmortem investigation during respiratory illness outbreaks. The different morphologic patterns of the lesions discovered in the lungs of Nilgai, who died of pneumonia, point to the participation of distinct combinations of infectious starting and compounding agents. The findings of this study showed different pneumonia phases. A significant capillary permeability with proteinaceous edema leaking into the alveoli and alveolar pneumocyte destruction is the first sign of acute serous interstitial pneumonia. According to van Dijk *et al.* (2017) acute fibrinous-necrotizing interstitial pneumonias may be caused by infectious hematogens that induce extensive fibrinous material exudates into the alveoli and fibrinoid necrosis of the alveolar septa.

Our study findings state and validate that, while Nilgai are Asia's largest antelopes, acute or subacute infections rarely afflict them, and epidemics are rare among their populations. However, it emphasizes that, in Nilgai, respiratory diseases are the most common cause of



death. This is consistent with the current study of Worku *et al.* (2016), which found that pneumonia and other serious respiratory issues were the main causes of mortality in the Nilgai subjects.

Moreover, according to our study's insights, various lesions are associated with respiratory diseases in Nilgai. These include emphysema, congestion, hydatidosis, and atelectasis. However, in our study most percentage of the lung emphysematous lesions were present throughout the lungs. Gross lesions are anomalies that primarily affect the caudodorsal lungs, parenchyma and pleural portion or the entire lung Rezac *et al.* (2014). In particular, Szczyrek *et al.* (2011) mentioned emphysema as a significant lesion resulting in chronic obstructive pulmonary disease and damage to the lung's air sacs. The presence of emphysema in the lungs of the studied Nilgai corroborates these observations and highlights the severity of respiratory complications in affected individuals. According to Chauhan *et al.* (2010), emphysema is characterized by the expansion of the alveoli and an increase in the volume of air in the lungs. It could be focal or widespread, acute or chronic. Its causes may include bronchitis, pneumonia, allergies, pulmonary adenomatosis, and atelectasis in the surrounding lung tissue. In addition, Marie (2016) discovered lungworms (0.44%) and pneumonia (0.66%) instances in its abattoir in the Iraqi province of Kerbala. The incidence of abscess, emphysema, pulmonary hydatidosis, congestion, atelectasis and lung parasites was also discovered by Tsegaye and his team during the course of their research (Tsegaye *et al.*, 2016). Also, an abattoir study in Northern Tanzania Swai *et al.* (2013) determined the prevalence of Contagious Bovine Pleuropneumonia (CBPP). However, Belkhiri *et al.* (2009) say that bovine pulmonary edoema cases are linked to Respiratory Syncytial Virus (RVS).

In this study, *Mycoplasma*'s were isolated from a startlingly high percentage of lungs, indicating that they are unquestionably crucial to the development of the disease. According to Islam *et al.* (2023), it appears that *Mycobacterium bovis* and *Pasteurella* spp. are associated with the development of the disease and need more investigation. Other bacteria are as likely as not crucial pathogens in the general pneumonic syndrome because they are much less prevalent. Although *Haemophilus* spp. were not identified in this study compared to some other studies, it's possible that the isolation methods were not sensitive enough to find a few of these organisms among other, faster-growing ones. Grey hepatization has been observed in this investigation, as well as in previous research, including the work conducted by Belkhiri *et al.* (2009). These outcomes differed significantly from those noted by Gourlay *et al.* (1970), where no grey hepatization was present. That could be the result of deoxygenating in our case, as lungs were emphysematous.

## 6. Conclusions

The findings of this study shed important light on the respiratory ailments of the giant antelope species Nilgai (*Boselaphus tragocamelus*). The investigation of a deceased male Nilgai revealed severe respiratory issues, with pneumonia brought on by *Pasteurella multocida* and *Mycoplasma bovis* being the primary cause of death. It is well recognized that these infections have a substantial part in the development of Bovine Respiratory Disease (BRD) in various animal species, including domestic cattle. These pathogens in the Nilgai's lungs indicate that they can seriously harm this species' respiratory system. The most challenging disease to treat when it worsens is pneumonia and respiratory illnesses. As a result, regular inspection and testing by veterinarians is a crucial step to control the disease because it has started to become

resistant to later-stage therapies. Nilgai populations in India that are free to roam likely contact illnesses from domestic animals. Sadly, these findings highlight some neglected issues of the hygiene of cattle and their products, as well as the health of humans in terms of personal hygiene and meat consumption. It has been discovered that the primary causes of pneumonic alterations and pulmonary lesions are primarily viral and bacterial illnesses. As a result, recommendations should focus on growing healthy cattle and utilizing regular veterinarian inspections.

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### **Ethical statement**

No animals were harmed in the course of this research study.

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