RESEARCH ARTICLE

Pathological Studies and Bacteriological Characterization of *Streptococcus* Species Associated with Pneumonia in Rabbits

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Abstract

Respiratory infections are major cause of death in rabbits, which are often caused by bacterial pathogens. The present study was conducted to determine the occurrence of streptococcal infections with pneumonia in rabbits through macroscopic, microscopic pathological and bacteriological examination. A total of 45 rabbits that died between October 2025 and February 2026 were brought for postmortem examination to the Department of Veterinary Pathology of the College in Rajendranagar, Hyderabad. Among them 29 were bunnies (2 to 6 months) and 16 adult rabbits (6 months to 3 years). The necropsy was conducted as per protocol in clean environment. 9 adult rabbits and 20 bunnies showed pneumonia lesions on postmortem examination. Necropsy findings showed consolidation in lungs, fibrinous exudation, abscess and pleuritis. Microscopic analysis of lung smears revealed Gram-positive cocci organized in chains. Histopathology of lungs revealed suppuration, inflammatory cells infiltration, emphysema and haemorrhage. The organism was isolated on Edwards medium and characterized by colony morphology and biochemical traits, including negativity for catalase, oxidase and positivity for bile esculin. These findings confirmed presence of *Streptococcal* species associated with pneumonic lesions in rabbits and emphasize the importance of bacteriological and histopathological investigations for accurate diagnosis of respiratory infections.

Keywords: Edwards medium, Gross pathology, Histopathology, Rabbit pneumonia, *Streptococcus* spp.

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Introduction

Pneumonia in rabbits is a prevalent infectious disease that causes considerable economic losses in commercial rabbitries and laboratory colonies. Several bacterial agents are involved in rabbit pneumonia, such as *Pasteurella multocida, Bordetella bronchiseptica, Pseudomonas* spp., *Staphylococcus aureus,* and *Streptococcus* spp., *(*Rougier *et al.,* 2006). *Streptococcus* species are Gram-positive cocci usually found in the normal digestive or respiratory tract in humans and in some animal species, including rabbits. In conditions of immunosuppression, it can cause serious infections that can be difficult to treat or can even lead to death if not treated properly (Iorgoni *et al.,* 2025). *Streptococcus* species, which are catalase-negative cocci that usually form chains and can cause suppurative and fibrinous inflammatory reactions (Yershov *et al.,* 2005). Rabbits with streptococcal infections may develop pneumonia, septicaemia, abscesses, or pleuritis, especially when they are stressed, crowded, or not getting enough air (Moody, 1916). For proper diagnosis, the pathological findings must be compared with bacteriological and biochemical evidence. The current study was designed to isolate and characterize *Streptococcus* spp. from the lungs of rabbits exhibiting pneumonic lesions.

Materials and Methods

Sample Collection and Preparation

A total of 45 rabbits that died between October 2025 and February 2026 were presented for postmortem examination to the Department of Veterinary Pathology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad (India). The necropsy was conducted as per the protocol in a clean environment (King *et al.,* 2014). During necropsy, the thoracic cavity was carefully opened and the lungs were examined for gross pathological lesions such as congestion, consolidation, fibrinous exudation, pleuritis, edema, and abscess. Lung tissues showing pneumonic lesions were collected aseptically in a sterile container for bacteriological analysis and sterile swabs were used to collect samples from

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affected lung tissue, which were immediately inoculated in nutrient broth initially. Affected lung tissues and other organs like liver were collected in sterile containers with 10% Neutral Buffered Formalin (NBF) for histopathological examination.

Examination of Gross Pathology

During necropsy the thoracic cavity was opened and carefully examined for colour, consistency, surface changes, presence of exudate and extent of consolidation. Gross lesions observed in the lungs and other organs were recorded and photographed.

Bacterial Cultural Examination

Lung samples from pneumonic lesions were swabbed and inoculated into a nutrient broth and incubated at 37°C overnight. A loopful of inoculum was taken and then streaked on to Edwards medium for selective isolation of *streptococci* species and the inoculated agar plates were incubated for 24-48 h at 37°C in an aerobic environment (Sanders, 2012).

Smears were made from lung exudate and cultured colonies, fixed with heat, and stained using the Gram staining method (Paray *et al.,* 2023). The stained slides were examined under oil immersion by light microscope to determine the morphology and Gram's reaction of the bacteria.

Biochemical Tests

Standard biochemical assays were conducted, including tests for catalase, oxidase, and bile esculin as per the standard procedure (Talaiekhozani, 2013). Identification of bacteria was accomplished through morphological, cultural, and biochemical tests.

Histopathology

Collected tissues were processed by routine paraffin embedding technique. Tissues sections of approximately 4-5 µm thickness were prepared by using a microtome and stained with hematoxylin and eosin (H&E) for microscopic examination as per the standard protocol (Slaoui *et al.,* 2017).

Statistical Analysis

The data obtained were compiled and analyzed using descriptive statistical methods. The occurrence of pneumonic lesions in rabbits was calculated as percentages based on the total number of rabbits examined. The results were expressed as percentages to determine the prevalence of pneumonic lesions associated with *Streptococcus* spp.

Results and Discussion

Prevalence

A total of 45 rabbit carcasses were examined during the study period. Among them, 29 rabbits (64.4%) showed pneumonic lesions on necropsy examination. Of these, 20 cases (68.96%) were observed in bunnies (2-6 months) and 9 cases (31.03%) were recorded in adult rabbits (6 months - 3 years) (Table 1). The higher susceptibility in younger animals may be attributed to their immature immune system and increased vulnerability to environmental stressors and opportunistic infections (Rougier *et al.,* 2006; Fernandez *et al.,* 2023).

Table 1: Age-wise occurrence of pneumonic lesions in rabbits

Age group	Total examined	Pneumonia cases	Percent
Bunnies (2-6 months)	29	20	68.96
Adults (6 mo-3 Yrs)	16	9	56.25
Total	**45**	**29**	**64.44**

Gross Pathology

Gross examination of the lungs revealed severe congestion with extensive areas of consolidation exhibiting a firm, liver-like consistency characteristic of hepatization. The affected pulmonary lobes were heavy, non-collapsing, and dark red to grey in appearance (Fig. 1). The fibrinous exudate was evident on the pleural surfaces, accompanied by marked pleural thickening suggestive of fibrinous pleuropneumonia. On the cut section, abundant suppurative inflammatory



Fig. 1: Thoracic cavity showing non-collapsed lungs in rabbits



Fig. 2: Lungs showing suppurative foci in rabbits.

exudate oozed out from the airways and parenchyma (Fig. 2). These pathological findings were consistent with acute fibrinopurulent pneumonia, reflecting a severe inflammatory response involving both the pulmonary parenchyma and pleural surfaces. Similar gross lesions have been reported in earlier studies on bacterial pneumonia in rabbits (Yershov *et al.,* 2005; Moody, 2016).

Isolation of *Streptococci* on Edwards Medium and Microscopic Findings

Inoculation and incubation of lung tissue on Edwards medium showed small, smooth, translucent to greyish colonies which were discrete, circular (Fig. 3). Visible growth was evident within 24 to 48 h of incubation, indicating active bacterial proliferation under the given culture conditions. These cultural features were consistent with the organisms belonging to the genus *Streptococcus*.

Gram-stained smears from lung and cultured colonies revealed Gram-positive cocci arranged predominantly in chains and occasionally in pairs. The bacteria appeared spherical to ovoid in shape and stained deep violet on Gram staining, confirming their Gram-positive nature. The organisms were non-motile and non-spore forming. The chain formation was due to division in a single plane, resulting in the typical "chain-like" arrangement observed under oil immersion microscopy. These microscopic features were consistent with the morphological characteristics of the genus *Streptococcus* (Fig. 4).

Biochemical Tests

The bile esculin test performed to determine the organism's ability to hydrolyze esculin in the presence of bile was positive suggesting that the isolate belonged to bile-esculin-positive *streptococci*, such as members of the Group D *streptococci*, thereby aiding in its biochemical identification. The oxidase test conducted to determine the presence of cytochrome C oxidase enzyme, and the catalase test performed to differentiate *Streptococcus* species from catalase-positive organisms such as *staphylococci*, revealed a negative reaction. These results confirmed the absence of the oxidase reaction and catalase enzyme, and were consistent with the biochemical characteristics of *Streptococcus* species. Biochemical tests in current study were in correlation with earlier research (Chuard and Reller *et al.,* 1998; Talaiekhozani, 2013).

Histopathology

Microscopic examination of lung sections revealed presence of suppurative mass (Fig. 5), infiltration of inflammatory cells, emphysema, edema, haemorrhage and congestion (Fig. 6).

Microscopic examination of liver sections showed homogenous pinkish suppurative mass, inflammatory cell infiltration, degenerated hepatocytes, sinusoidal haemorrhage, bile duct proliferation dilated and congested central vein (Fig. 7, 8)

Histopathological findings indicated severe pulmonary tissue damage due to bacterial infection and were in accordance with previous reports of *streptococcal* pneumonia in rabbits (Herting *et al.,* 1997; Vishwas *et al.,* 2000; Yershov *et al.,* 2005). Microscopically the presence of Gram-positive cocci organized in chains is a critical diagnostic characteristic that differentiates *Streptococcus* from *Staphylococcus*, which are found in clusters and exhibit catalase positivity (Quinn *et al.,* 2011; Markey *et al.,* 2013). Growth on Edwards medium further supported the identification, as this selective medium facilitates differentiation of streptococci based on colony morphology while the negative catalase and oxidase reaction provided additional confirmation of the isolate's identity (Sanders, 2012).

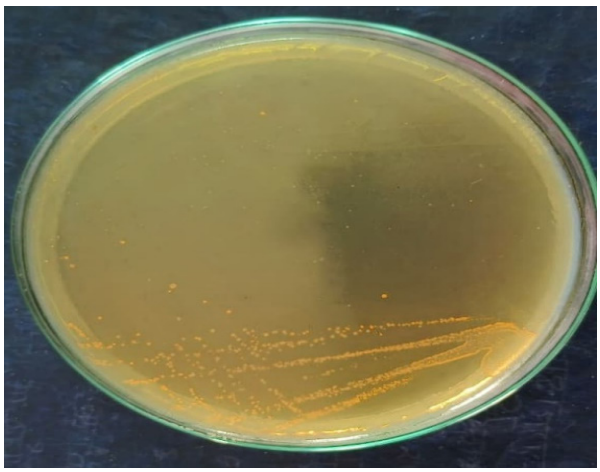


Fig. 3: Small, smooth, translucent colonies of *streptococci* on Edwards medium

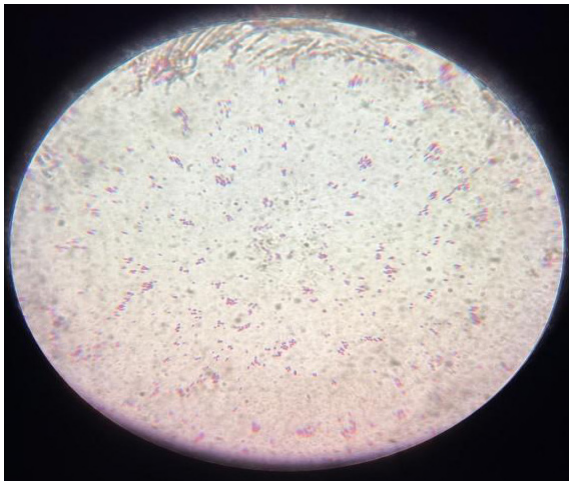


Fig. 4: Gram positive cocci arranged in chains in Gram-stained smears from lung

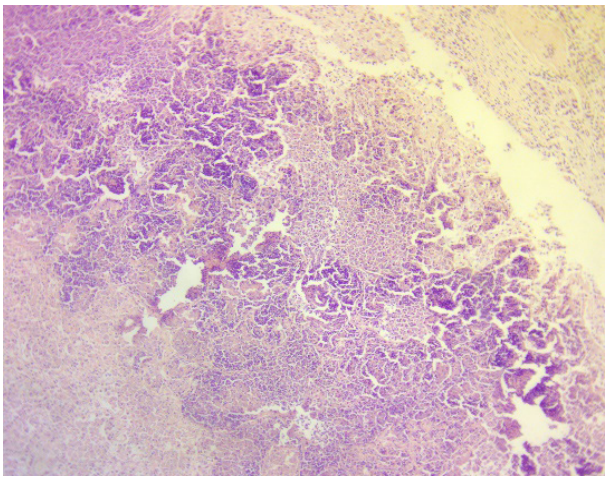


Fig. 5: Histopathology of rabbit lung showing suppurative mass

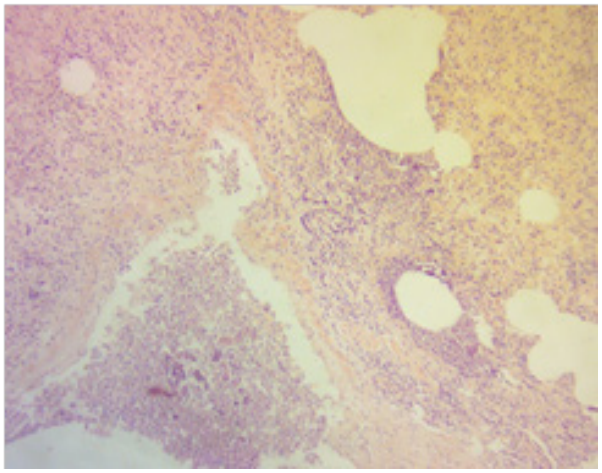


Fig. 6: Lung showing inflammatory cells infiltration, emphysema, edema, haemorrhage & congestion

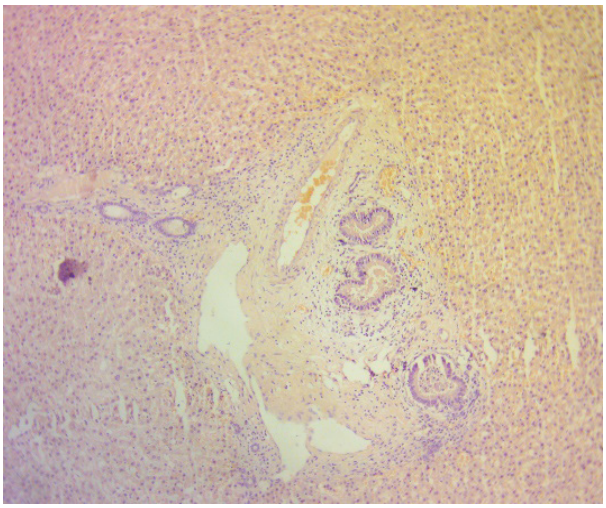


Fig. 7: Liver showing bile duct proliferation, degenerated hepatocytes, sinusoidal haemorrhage, congested central vein

In rabbits, streptococcal pneumonia is often associated with predisposing factors such as environmental stress, poor ventilation, or concurrent infections that increase susceptibility to opportunistic pathogens (Rougier *et al.,* 2006; Iorgoni *et al.,* 2025).

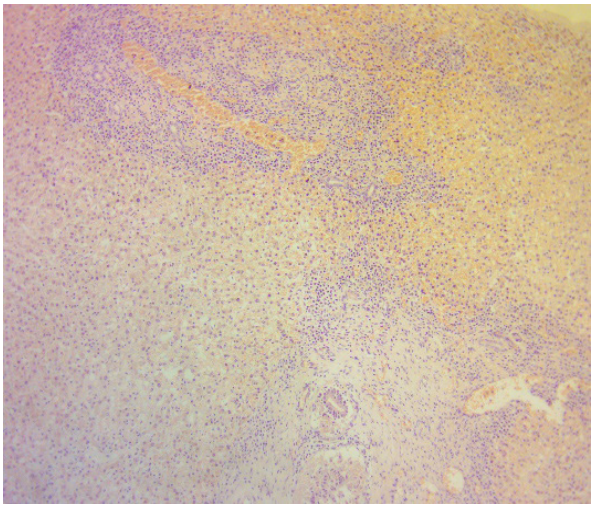


Fig. 8: Rabbit liver showing pinkish suppurative mass, sinusoidal haemorrhage, inflammatory cell infiltration

Conclusion

The present study confirmed the occurrence of *Streptococcal* infections associated with pneumonic lesions in rabbits, with higher prevalence in young bunnies compared to adult rabbits. The diagnosis was established based on characteristic gross and histopathological findings, including consolidation and fibrin deposition, along with microscopic observation of Gram-positive cocci arranged in chains. Histopathological examination further substantiated the diagnosis. The findings highlight the significant role of *Streptococcus* spp. as an important bacterial pathogen contributing to respiratory disease in rabbits. Further confirmation was obtained through successful growth of bacteria on Edwards medium and a biochemical profile. Collectively, these findings confirmed the occurrence of *streptococcal* pneumonia and highlight the importance of systematic bacteriological investigation in the accurate diagnosis and management of respiratory diseases in rabbits. Improved management practices, including proper ventilation, hygiene and stress reduction are essential to minimize the risk of such infections in rabbit populations.

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