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## A rare case report of *Pasteurella multocida* infective valvular endocarditis in a laboratory rabbit

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**Abstract**

*Pasteurella multocida* is a gram negative bacterium and an inhabitant of the oral flora of a variety of livestock and domestic animals. Although sepsis and pneumonia by *P. multocida* infection is common in rabbits, infective endocarditis is extremely rare. There are limited reports of *P. multocida* infective endocarditis in humans, but no such report has been published in rabbits, so far. The case is reported of a dead adult male New Zealand White rabbit infected with sub-clinical Pasteurellosis. Necropsy findings revealed gangrenous pneumonia and vegetative endocarditis. Culture and staining examination revealed the presence of *Pasteurella multocida*. The remaining rabbits from the adult stock were found with sneezing, nasal discharge, conjunctivitis, dyspnoea and pyrexia. Antibiotic sensitivity test of isolated *Pasteurella multocida* showed higher sensitivity to ceftiofur. The rabbits showed rapid recovery from the infection after treatment with ceftiofur sodium.

**Keywords:** Rabbit, *Pasteurella multocida*, pneumonia, endocarditis, septicemia, ceftiofur

**1. Introduction**

Pasteurellosis is one of the most common bacterial diseases of rabbits affecting respiratory system with considerable economic loss in large production units throughout the world (Takashima *et al.*, 2001) [1]. The gram negative bacterium occurs as a commensal in nasopharynx and digestive tract. The infection known as, Pasteurellosis is characterised by various clinical symptoms, such as, respiratory distress, conjunctivitis, otitis, sinusitis, abscesses and septicaemia, but infection by *P. multocida* can also appear sub-clinical that is infected rabbits do not show symptoms as long as the immune system is functioning normally or until they stressed (DeLong and Manning, 1994) [3]. Often, pasteurella occurs with other bacteria, simultaneously causing infection. Pasteurella colonizes the nasal cavity, infection may then spread into the sinuses and facial bones and spread through the Eustachian tubes to the ears, through the nasolacrimal duct to the eye, through the trachea to the lower respiratory tract, and through the blood stream to joints, bones, and other organ systems. Damage to the nasal cavity can be permanent (atrophic rhinitis), impairing local defence mechanisms and allowing reinfection with pasteurella or other bacteria (Chrisp and Foged, 1991) [2]. The variability in clinical signs as well as the pathogenicity of the disease may be influenced by different virulence factors of *P. multocida* such as a capsule, fimbriae, lipopolysaccharides (endotoxin), dermonecrotoxin, neuraminidase etc. (Glorioso *et al.*, 1982; Rhoades and Rimler, 1987; Straus *et al.*, 1996) [5, 9, 10]. Snuffle is a general term describing a group of upper respiratory disease symptoms due to bacterial infection such as *Pasteurella multocida*, *Bordetella bronchiseptica* and *Staphylococcus sp.* in rabbits. The *P. multocida* affected rabbits are often the chronic snufflers due to reinfection (Premalatha *et al.*, 2009) [9].

**2. Case history and clinical observations**

Ten adult New Zealand White rabbits from the adult stock of private research organization from Chennai were found ill with the symptoms of sneezing, mucus discharge from the nasal cavity with soiling of the hair around nose (Fig.1) and forepaw and pyrexia with rectal temperature ranging from 39.6°C to 40.8°C. Four rabbits showed conjunctivitis and mucopurulent discharge from the eyes (Fig.2). Symptomatic treatment such as chlorpheniramine maleate syrup, Neomycin ophthalmic ointment and intramuscular injection of Meloxicam were given by the attending Veterinarian. Bacterial infection was suspected and nasal swabs and eye swabs from the affected animals were collected and sent to the Microbiology unit for bacteriological examination to identify the causative organism and advised to carryout.

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Antibiotic sensitivity test (ABST), for the isolated organism to select the suitable antibiotic for treatment regimen. The next morning, one rabbit showed signs of dyspnoea, anorexia, abdominal breathing and the rectal temperature of 41.5°C, the next day it was found dead and a necropsy was carried out.



**Fig 1:** Mucus discharge from the nasal cavity

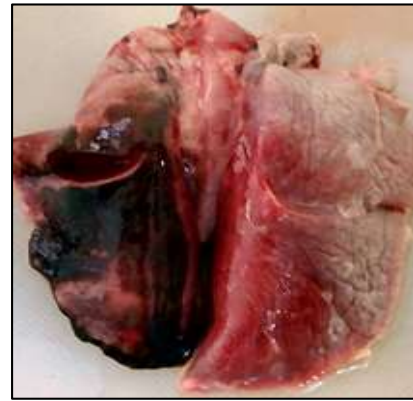


**Fig 2:** Ocular discharge

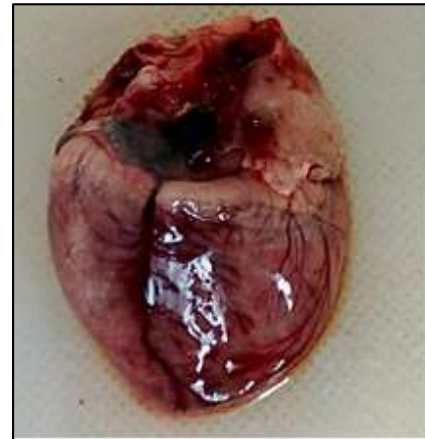
### 2.1 Pathological findings

The necropsy findings revealed congested nasal mucosa and accumulation of mucus in the nasal cavity. Severely congested pharynx and larynx mucosa, haemorrhagic tracheitis characterized by inflamed and hardened trachea with petechial and ecchymotic haemorrhages and hyperaemia of the mucosa. Lungs showed severe inflammation, haemorrhagic pneumonia in left lung and necrotizing gangrenous pneumonia in right lung. Right lung was severely necrotized, dark in colour and hard in consistency (Fig.3). Accumulation of serosanguineous exudate was found in the thoracic cavity. Enlarged and dark colored mediastinal lymph nodes were noticed.

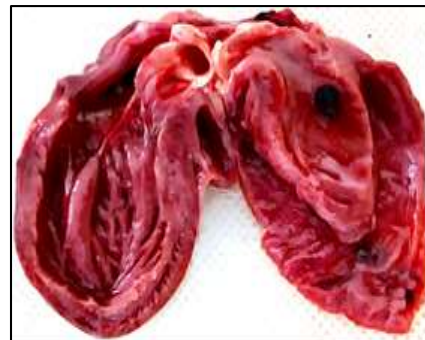
The pericardium was thickened and adhered to the epicardium. The heart was enlarged (cardiomegaly) and congested (Fig.4). The cardiac valves were severely thickened and small white fibrinonecrotic masses (vegetative valvular endocarditis) were present in the adjacent wall (Fig.5). Aorta and pulmonary artery were inflamed and hard in consistency. The lesions revealed vegetative endocarditis. Liver and spleen were congested. Petechial haemorrhages were found on gastric mucosa. Tissue samples were collected in 10% neutral buffered formalin and processed for histopathological examination.



**Fig 3:** Haemorrhagic and gangrenous pneumonia

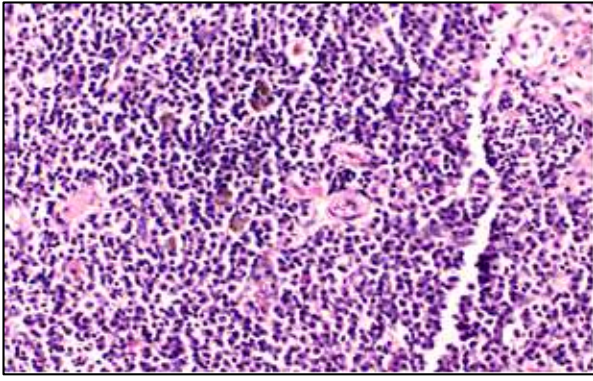


**Fig 4:** Enlarged and congested heart



**Fig 5:** Vegetative valvular endocarditis

The histopathological examination of lungs showed oedema, congestion, multifocal to diffuse necrosis and haemorrhage, severe infiltration of the inflammatory cells and necrotic debris in bronchiole (Fig.6). Alveoli were highly infiltrated with macrophages and lymphocytes along with some neutrophils. Bronchiolar epithelium was characterized by hypertrophy and accumulation of lymphocytes and plasma cells. Multifocal necrosis, oedema and infiltration of lymphocytes and macrophages were observed in the tracheal lamina propria. Liver and spleen exhibited congestion, necrosis of hepatocytes and infiltration of lymphocytes. Microscopically there were no appreciable lesions in kidneys. Other organs like gastric mucosa and meninges showed minimal congestion, diffuse haemorrhages and minimal infiltration of inflammatory cells.



**Fig 6:** Lung showing severe infiltration of the inflammatory cells (H&E 20X).

The microscopic changes in the heart comprised of diffuse necrosis and haemorrhages and in the valves, heavy infiltration of neutrophils surrounded by fibroblasts and foamy macrophages. The bacterial colonies had a cloudy appearance, and separate bacterial cells were not visible.

## 2.2 Bacteriological examination

For primary isolation of the organism, swabs taken from the lung, heart, and trachea of the dead animal and from the nasal cavity and eye of the live animals were inoculated on nutrient agar and blood agar supplemented with 7% sheep blood. The plates were incubated under aerobic conditions for 24-48 h at 37 °C. Identification and confirmation of bacterial species was assessed by observation of the colonial morphology, Gram's staining and by biochemical methods. After 48 h of incubation, grey to grey-white large mucoid, non-hemolytic colonies were observed on the blood agar. The heart blood smear, liver impression smear and smears prepared from colonies stained with Leishman's stain revealed bipolar *Pasteurella multocida* organisms and Gram's staining revealed the gram negative rods. In biochemical tests, the organism produced oxidase, catalase, indole and presence of ornithine decarboxylase that fermented mannitol, produced acid by fermentation of glucose and no gas production, confirmed *Pasteurella multocida*. Antibiotic sensitivity test (ABST) was performed for the isolated organism by the Kirby-Bauer disk diffusion method (NCCLS, 1997) [6]. The ABST results showed higher sensitivity to ceftiofur followed by enrofloxacin, gentamicin, chloramphenicol and tetracycline and resistant to ampicillin, neomycin and azithromycin.

## 3. Treatment

The rabbits were treated with intramuscular injection of ceftiofur sodium at a dose of 2.2 mg/kg once daily for seven consecutive days and chlorpheniramine maleate @ 1mg/kg b.wt orally. The discharges from the eyes were cleaned with sterile cotton and 0.3% gentamicin sulfate ophthalmic solution was applied in both the eyes twice daily to treat conjunctivitis. Liver tonic containing B-complex vitamins was given as a supportive therapy. The symptoms were improved gradually by the third day of initiation of treatment. All the animals recovered completely after treatment with ceftiofur sodium. Nasal swabs showed negative results in culture examination.

## 4. Discussion

Though sepsis and pneumonia by *P. multocida* infection is common in rabbits, infective endocarditis is extremely rare

and it is difficult to diagnose this condition in the live animals due to lack of diagnostic facilities like ECG and echocardiography in the animal houses or rabbitries. In the presented case, the infection was sub-clinical; the animal was healthy and free from any of the clinical signs till the terminal stage. It was in agreement with the statement by Di Giacomo *et al.* (1983) [4]. The Clinical signs observed in this report *viz.* nasal discharge, pyrexia, sneezing, conjunctivitis, dyspnoea and abdominal breathing were also reported by Premalatha *et al.* (2009) [8] in their report of incidence of pasteurellosis in a rabbit farm. The reported post mortem and histopathology findings such as haemorrhagic pneumonia, liver and splenic congestion, tracheitis etc. were opined with the findings of Patel *et al.* (2016) [7] who studied the clinical pathology of experimentally induced *Pasteurella multocida* B:2 infection in rabbits. The antibiogram of culture showed higher sensitivity to ceftiofur followed by enrofloxacin, gentamicin, chloramphenicol and tetracycline and resistant to ampicillin, neomycin and azithromycin. This is on par with the findings of Cuevas *et al.* (2020) [1] who reported that *P. multocida* type B isolates from pigs and cattle with acute septicaemia showed 98% susceptibility to ceftiofur followed by 86% to enrofloxacin, 84% to gentamicin, 81% to chloramphenicol. Hitherto, the incidence of *Pasteurella multocida* infective vegetative valvular endocarditis in rabbits was not reported by any of the earlier authors, the presented report is the first of its kind.

## 5. Conclusion

Complete elimination of the *Pasteurella* organisms is difficult since they occur as commensal in nasopharynx and digestive tract. It was advised to provide better ventilation, adequate floor space, proper cleaning of cages with disinfectant, provision of autoclaved feed and purified drinking water, usage of personal protective equipments, isolation and treatment of the infected animals and hygienic management of animal facility premises to prevent the incidence and spread of bacterial infections.

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