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A case report-isolation and identification of *Rhodococcus equi* from heart blood and tissue samples in foal

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Abstract

In present study multifocal to coalescing nodules, containing a yellowish white creamy material were the predominant lesions found in lungs of *Rhodococcus equi* infected foal. Colonies of *Rhodococcus* were Pin-point, Translucent, smooth, shiny and nonhemolytic on blood agar, but became larger, shiny, mucoid, tends to merge and salmon-pink in color with age. *Rhodococcus* is appear as a gram positive cocci or rod.

Keywords: *Rhodococcus equi*, Foal, nodule, cocci, rods

Introduction

Rhodococcus equi is the most serious cause of pneumonia in foals. The most common clinical manifestation of *R. equi* infection is chronic pyogranulomatous bronchopneumonia with abscessation and associated suppurative lymphadenitis. In addition to pulmonary disease, extrapulmonary disorders may result from infection.

It is known for zoonotic infections in foals that are between 1 to 4 months of age. It is ranked among the top most important pathogens in the horse industry especially because of its high prevalence and mortality rate (Khurana *et al.* 2009) [4].

R. equi can appear as a Gram-positive coccus or as a rod. It is capsulated and sometimes weakly acidfast (Quinin & carter, 2004) [5]. It is facultative intracellular pathogen, that is nearly ubiquitous in soil. Inhalation of dust particles laden with virulent *R. equi* is the major route of pneumonic infection (MSD Manual, 2015). Development of clinical disease is related to immunocompetency of individual foals; foals that produce little to no detectable γ interferon (IFN- γ) are at risk of developing pneumonia. Manure from pneumonic foals is a major source of virulent bacteria contaminating the environment. Foals with pulmonary infections swallow sputum laden with *R. equi*, which readily replicates in their intestinal tract. The pathogenicity of *R. equi* is linked to its ability to survive intracellularly, which hinges on failure of phagosome-lysosomal fusion in infected macrophages and failure of functional respiratory burst upon phagocytosis of *R. equi*.

R. equi infection is slowly progressive, with acute to subacute clinical manifestations. Clinical signs of disease are difficult to detect until pulmonary infection reaches a critical mass, resulting in decompensation of the foal. Pulmonary lesions are relatively consistent and include subacute to chronic suppurative bronchopneumonia, pulmonary abscessation, and suppurative lymphadenitis. At the onset of clinical signs, most foals are lethargic, febrile, and tachypneic. Diarrhea is seen in one-third of foals with *R. equi* pneumonia and may be caused by colonic microabscessation. Cough is a variable clinical sign; purulent nasal discharge is less common.

Material and Method

A foal suffered from severe respiratory problem was admitted to polyclinic, Udaipur from Fateh stud farm Kelwa, Rajsamand (Rajasthan), which was died. Post mortem was conducted by the team of RDDC, Udaipur & polyclinic, Udaipur to find out the cause of death. Heart blood sample, peritoneal cavity fluid and Swab from lung nodules were collected for isolation and identification of bacteria. These samples than inoculated into BHI Broth in Laboratory laminar flow and incubate for 6-8hr at 37 °C temp. After thata loopful culture was inoculated on blood agar and Mac Conkey Agarby streaking plate method and incubate aerobically for 24-48hr (Quinin & carter, 2004) [5]. Impression smears from lungs were also taken. These smears were stained with gram's stain & subjected to direct microscopy for presence of Bacteria).

After culture & identification of bacteria a Slant and Culture was also send to NRCE for further confirmation.

Result and Discussion

Post Mortem Findings

At necropsy, the foals' body condition was fair. Pulmonary lesions were consisted of multifocal to coalescing nodules ranging from a few millimeters up to 6 cm in diameter, containing a yellowish white creamy material, accompanied by consolidation. Fluid in peritoneal cavity. The lungs were also diffusely non-collapsed with rib impressions and elastic consistency. These findings are in correlation with Bonnie R. Rush (2015)^[1].

Gram positive capsulated rods were seen in the impression smears of lung.

Culture and Isolation

After 24 hrs incubation, the blood agar plate was examined for growth of bacteria and observe the colony characteristics and morphology. The bacterial isolates showed Pin-point, Translucent, smooth, shiny and nonhemolytic colonies, but became larger, shiny, mucoid, tends to merge and salmon-pink in color with age (after 7 days incubation). Initial confirmation of the isolates as *R. equi* was done by Culture characteristics, demonstration of the typical cellular morphology in Gram-stained smears and primary characterization tests. Rabya Javed *et al.* (2012)^[6] also reported similar colony characteristics of *Rhodococcus* bacteria. Similar findings were also observed by Hareton *et al.* (2018)^[3].

No growth on Mac Conkey Agar

Primary characterization tests findings Catalase- Positive, oxidase- Negative, Unreactive on HL medium are similar to Quinin & Carter (2004)^[5].

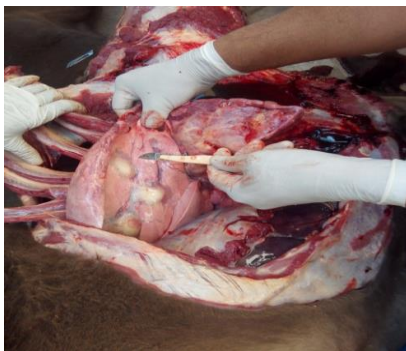


Fig 1: Multiple pulmonary abscesses characteristic of *Rhodococcus equi*



Fig 2: Cut section of lung nodule containing yellowish white creamy material

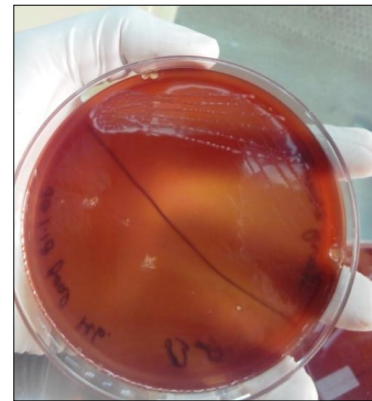


Fig 3: Pin-point, Translucent, smooth, shiny and no hemolytic colonies of *Rhodococcus equi* on Blood agar after 24hr incubations

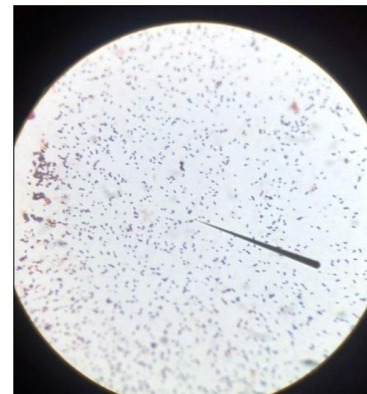


Fig 4: Photomicrograph showing gram positive coccobacilli *Rhodococcus equi*. Gram's staining.



Fig 3: Shiny, mucoid, tends to merge, Salmon pink coloured colonies of *Rhodococcus equi* on Blood agar after 7 days incubations



Fig 4: Photomicrograph showing gram positive rods of *Rhodococcus equi* Gram's staining. After 7 days of incubation

Conclusion

This study reveals clinical manifestation of *Rhodococcus equi* infection is closely associated with pneumonia and is confirmed by Post Mortem findings, culture and isolation of organism. In addition to pulmonary disease, extrapulmonary disorders exist as a result from secondary microbial infections.

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