CASE REPORT

RHODOCOCCUS EQUI – AN EMERGING HUMAN PATHOGEN IN IMMUNOCOMPROMIZED HOSTS: A REPORT OF FOUR CASES FROM MALAYSIA

SD Puthucheary,[1] V Sangkar[2], Asma Hafeez,[1] R Karunakaran[1], Nadeem S Raja[1] and HH Hassan[1]

Departments of [1]Medical Microbiology, [2]Medicine, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Abstract. *Rhodococcus equi,* a recognized pathogen in horses, is emerging as a human opportunistic pathogen, especially in immunocompromized hosts. We describe four immunocompromized patients who had serious *R. equi* infections with an overall mortality of 75%. The natural habitat of *R. equi* is soil, particularly soil contaminated with animal manure. Necrotizing pneumonia is the commonest form of infection but extrapulmonary infections, such as wound infections and subcutaneous abscess, have also been described in humans. *R. equi* is cultured easily in ordinary non-selective media. Large, smooth, irregular colonies appear within 48 hours. It is a facultative, intracellular, nonmotile, non-spore forming, gram-positive coccobacillus, which is weakly acid-fast staining and bears a similarity to diphtheroids. It forms a salmon-colored pigment usually after 48 hours incubation. A particular characteristic of this organism is that it undergoes synergistic hemolysis with some bacteria on sheep blood agar. *R. equi* may be misidentified as diphtheroids, *Mycobacterium* species, or *Nocardia*. *In vitro R. equi* is usually susceptible to erythromycin, ciprofloxacin, vancomycin, aminoglycosides, rifampin, imipenem and meropenem. The organism can be difficult to eradicate, making treatment challenging. Increased awareness of the infection may help with early diagnosis and timely treatment.

INTRODUCTION

Rhodococcus equi, previously known as "*Corynebacterium equi*", a gram-positive, weakly acid-fast staining coccobacillus, once thought to be exclusively an equine pathogen, is an emerging life-threatening opportunistic pathogen in immunosuppressed human hosts.

Primarily it causes zoonotic infections affecting horses, cattle, sheep and swine, causing pneumonia, lymphadenitis and pyometra. It is a rare opportunistic human pathogen causing pneumonia and pulmonary abscesses in patients with lymphoreticular malignancies and solid organ transplants (Van Etta *et al*, 1983). More recently, in the past decade, HIV infection has been associated with *R. equi* infection (Muntaner *et al*, 1997). Almost all human infections have occurred in patients who have defects of cell-mediated immunity with or without histories of animal exposure.

R. equi is readily found in soil, especially where domesticated livestock graze (Prescott, 1991). Intestinal carriage in adult herbivores is passive and only represents acquisition from contaminated grass. Exposure to soil contaminated with manure is the most likely route of acquiring infection in both animals and humans. Inhalation of dust particles laden with virulent *R. equi* is the major route of pneumonic infection, but infections by the oral route (due to ingestion of soil or food) or by direct inoculation due to trauma have also been described (Van Etta *et al*, 1983). Necrotizing lobar pneumonia is the commonest form of infection caused by *R. equi.*

Extrapulmonary manisfestations described in human beings include subcutaneous nodules,

Correspondence: Professor SD Puthucheary, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
Tel: ++603-7967 6674; Fax: ++603-7958 4844
E-mail: puthu@ummc.edu.my, puthu@um.edu.my

brain and renal abscesses, lymphadenitis, endophthalmitis and osteomyelitis (Mayor *et al*, 1995) and fever of unknown origin. Bacteremia and dissemination of infection follow from the primary site of infection which usually is the lung. Numerous complications are related to *R. equi* infections and the reported mortality is 50-55% in patients infected with HIV and 20-25% in patients with non-HIV associated immunocompromized conditions (Harvey and Sunstrum, 1991).

We report four cases of *R. equi* infection in immunocompromized hosts seen at the University Hospital, Kuala Lumpur, between November 2003 and February 2005. We believe this is the first report of such infections in Malaysia. This article aims to bring about improved recognition of this easily overlooked pathogen.

CASE HISTORIES

The relevant clinical data of the four patients with *R. equi* infections are summarized in Table 1. All four patients were adults, the youngest being 16 and the oldest, 60 years. There were three males and one female, who was a Thai national.

Fever, cough and signs and symptoms relevant to the respiratory system were the predominant presenting features. Immune system dysfunction was present in all four cases; one had AIDS and the three others had non-HIV associated immunocompromized conditions. All had very low peripheral white cell counts. All four patients were given empirical antimicrobial therapy. Two patients (cases # 3 and 4) were given specific antimicrobials after culture and

Table 1
Summary of findings in 4 cases of *Rhodococcus equi* infection.

No.	Age (yr) Sex	Clinical presentation	Underlying illness	Chest X ray	Antimicrobial therapy	Duration of illness
1	60 M	Cough, fever, lethargy, abdominal swelling, anorexia-2 months. Bilateral coarse crepitations, Pleural effusion R lung, Hb= 8.2 g/l TWBC= 0.6 x 10^9/l	Acute lymphatic leukemia	Multiple bilateral patchy opacities. Consolidation, effusion of R. lower lobe	Cefepime- 2 wks Piperacillin-tazobactam	2 months
2	16 M	Productive cough, greenish sputum, fever 7 days, lethargy, pallor, Hb= 8.7 g/l TWBC= 0.9 x 10^9/l	T-cell lymphoblastic leukemia	NA	Piperacillin-tazobactam, Gentamicin, Cefepime	1 week
3	29 F	Cough, fever, chill, rigors, cachexia, tachypnea. Oral thrush R. inguinal node +ve Hb=12 g/l TWBC= 1.8 x 10^9/l	AIDS	Cavitation. Consolidation of R. lower lobe	Ceftriaxone, Fluconazole Vancomycin, Azithromycin HAART	4 weeks
4	54 M	Cough, fever, pallor, breathlessness. Coarse crepitations Hb=10 g/l TWBC=0.6x10^9/l	Old PTB, diabetes, congestive cadiac failure	Consolidation, Fibrosis of L. lower lobe CT scan-necrosis of mediastinal lymph nodes	Azithromycin, Flucloxacillin	5 weeks

NA = not available; R = right; L = left

sensitivity tests were available. *R. equi* was isolated from the blood cultures of the first three patients and from a bronchoalveolar lavage of the fourth patient. Patients number one, three and four died, but the status of number two was not available at the time of writing this paper.

We were unable to elicit any history of exposure to horses or to contaminated soil, but the first patient lived in a village and tended his garden frequently.

The following is a more detailed report of case number one.

Case # 1

A 60 year old man was admitted to the University Hospital on 18 November 2003 with a history of cough, fever, lethargy, anorexia, loss of weight and abdominal swelling of two months duration. The swelling was not painful. He had been admitted to another hospital one month previously for a biopsy but did not follow-up there. On examination, he was pale, jaundiced, cachexic and dehydrated. His blood pressure was 90/50. His lungs were clear. There was no discernible lymphadenopathy peripherally. A large ill defined hard mass was palpable in the abdomen, about 30 cm by 30 cm which was mildly tender.

A CT scan of the thorax, abdomen and pelvis showed enlarged lymph nodes in the aortopulmonary window, subclavian area and markedly enlarged mesenteric nodes. The lung parenchyma was clear but there was atelectasis at the bases. Ascites were present. Biochemically it was noted that he had a metabolically active lymphoproliferative disorder with a markedly raised lactate dehydrogenase. A bone marrow examination confirmed the diagnosis of acute lymphoblastic leukemia.

On the 26th of November he developed erythema over the left brachial region, fever and crepitations in the lungs. Blood cultures grew *Staphylococcus aureus* thought to originate from an infected PICC site. He was given cephipime for 10 days.

Following this he again developed bilateral basal crepitations, fever, breathlessness, with poor air entry, especially in the right lower zone. Chest X ray showed multiple bilateral patchy opacities with consolidation and pleural effusion of the right lower lobe (Fig 1). A pleural tap was carried out on the 15th of December and the fluid was found to be exudative with a WBC count of 48/µl. His blood culture taken on the same day grew *R. equi*. He was given piperacillin-tazobactam for nine days, then discharged on the 2nd of January 2005, to be followed up as an outpatient, but he died soon after. The final diagnosis was acute lymphatic leukemia with *R. equi* septicemia and pneumonia.

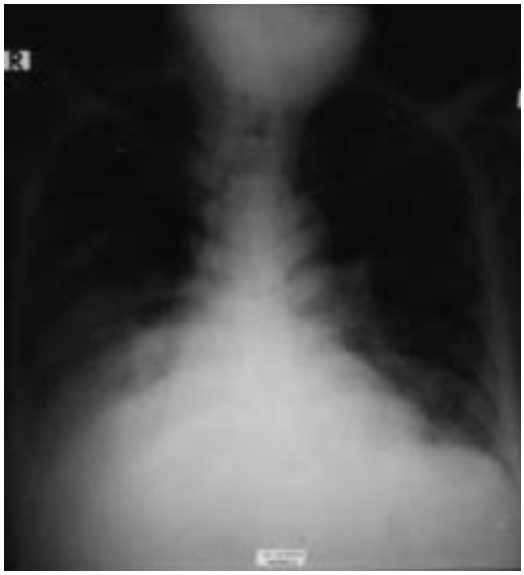

Fig 1–Chest X ray showing multiple patchy opacities with consolidation and pleural effusion of the right lower lobe.

DISCUSSION

The role of *R. equi* as a human pathogen was not established until 1967, when the first case report was published of a 29 year old man with plasma cell hepatitis who developed a cavitatory pulmonary lesion after cleaning animal pens at a stockyard (Golub *et al*, 1967). All three of our patients were immunocompromized with very low white cell counts. Patient number three had AIDS and a CD_4 count of zero. Current opinion is that the *R. equi* infection should be considered an AIDS-defining event (Albrecht, 1997). Therefore, *R. equi* should form part of the differential diagnosis of cavitary pneumonia in

patients with HIV infection.

Humans usually acquire infection by inhalation of soil contaminated with the manure of herbivores resulting in pulmonary infections but disease associated with the gastrointestinal tract without pulmonary involvement suggests ingestion of contaminated material as a possible route (Verville *et al*, 1994). Usually, no history of exposure or contact with farm animals is elicited, as was the case in our four patients. Delay in diagnosis often results because of insidious onset of disease, clinical similarity to mycobacterial and fungal infections, and the indistinguishable morphology of *R. equi* from normal diphtheroid respiratory flora (Linder, 1997).

Bacteriology

R. equi is a soil organism with simple growth requirements. It is cultured easily on ordinary nonselective media when incubated aerobically at 37ºC. Large, smooth, irregular, highly mucoid colonies appear within 48 hours. Although *R. equi* is named for its production of red pigment, cultures less than four days old rarely appear pigmented. After 4-7 days incubation, colonies usually develop a delicate salmon pink color, although they may be nonpigmented or slightly yellow (Scott *et al,* 1995). It is a facultative, intracellular, non-motile, non-spore forming organism. Gram stain shows pleomorphic gram-positive rods varying from coccoid to long, curved, and clubbed forms (Fig 2). The organism may be inconsistently acid-fast with Ziehl-Neelsen staining, depending on the age of culture and growth media. *R. equi* is non-fermenting (distinguishing it from pathogenic corynebacteria), gelatinase-negative, catalase-positive, usually urease-positive and oxidase-negative (Verville *et al*, 1994). Also helpful in identifying *R. equi* is synergistic hemolysis (resembling the CAMP test), displayed by cross-streaking on sheep-blood agar with a number of other bacteria, including *Arachanobacterium haemolyticum* and *Staphylococcus aureus* (Fig 3). In the modern microbiology laboratory, practical identification of *R. equi* is most easily accomplished by the application of a commercially available panel of biochemical tests (API Coryne, bioMerieux, Marcy-Etoile, France; Remel RapiD CB Plus System, Lenexa. KS, USA) to isolates which have the typical colony characteristics and Gram-stain morphology (Verville *et al,* 1994).

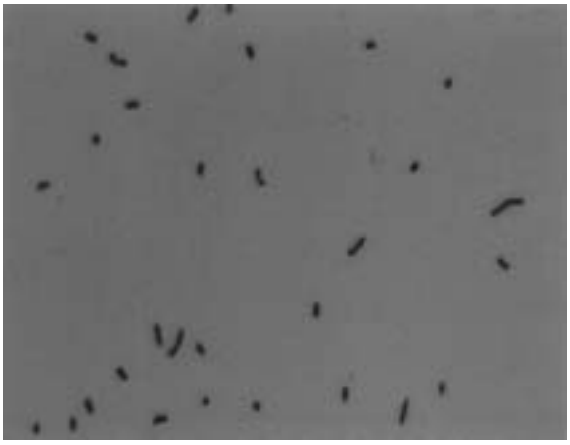

Fig 2–Coccobacillary forms of *R. equi* (Gram stain of 48-hour culture on sheep blood agar).

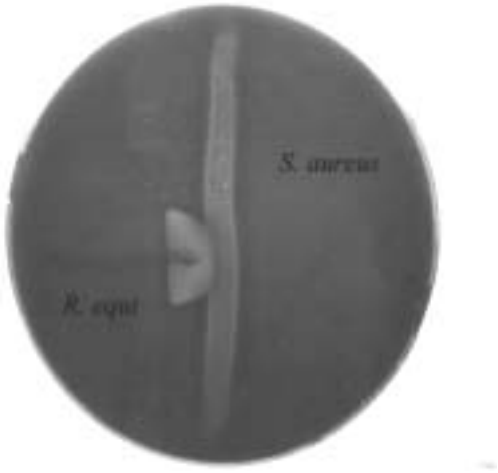

Fig 3–Synergistic hemolysis on sheep blood agar.

Virulence

The ability of *R. equi* to remain inside macrophages, grow and ultimately destroy the macrophages, is the property most closely associated with virulence in both the human and animal host (Hondalus and Mosser, 1994). Electron microscopic examination of cultured equine macrophages showed that organisms evaded killing by preventing phagosome-lysosome fusion, thus multiplying in and eventually killing the phagocytes (Zink *et al,* 1987). Soluble cytotoxic

substances were found to be associated with virulent phenotypes of the organism but these have not been characterized yet. Strains of *R. equi*, irrespective of virulence, produce cholesterol oxidase, which is responsible for the organism's participation in synergistic hemolytic reactions with other bacteria, such as *S. aureus. S. aureus* was isolated together with *R. equi* from the blood of patient number one and from the bronchoalveolar lavage of patient number four. Whether the synergistic hemolytic reaction seen *in vitro* plays any role *in vivo* in the pathogenesis of the infection is yet to be elucidated. Experiments using cultured mouse macrophages with phagocytosed *R. equi* suggest a role for cholesterol oxidase in macrophage destruction in infections (Linder, 1997).

In vitro, R. equi is usually susceptible to erythromycin, ciprofloxacin, vancomycin, aminoglycosides, rifampin, imipenem and meropenem. The intracellular survival of the organism has led to recommendations that *R. equi* infections be treated with lipophilic antibiotics that penetrate cells. Combined antimicrobial therapy involving parentral glycopeptide plus imipenem for at least three weeks, followed by an oral combination of rifampin, plus either macrolides or tetracycline has been recommended (Linder, 1997). Most authors recommend therapy be continued for a minimum of two months due to the frequency of relapses following shorter courses (Verville *et al*, 1994). However, host-immune competence may be the most important consideration in determining duration of therapy.

R. equi is a unique opportunistic pathogen in humans and an intact cell-mediated immunity appears to have a primary role in protection against infections. Recent investigations have shown that T-cell subsets, specifically functional CD4+ lymphocytes are necessary to effect complete clearance of a *R. equi* challenge in immunodeficient mice (Kanaly *et al*, 1996).

In summary, *R. equi* infection is an uncommon opportunistic infection in immunocompromized individuals. A high index of suspicion is required to diagnose pulmonary infections. The practice of regarding coryneform organisms in sputum Gram stains and cultures as contaminants may contribute to a significant number of missed diagnoses (Verville *et al,* 1994).

REFERENCES

Albrecht II. Redefining AIDS: towards a modification of the current AIDS case definition. *Clin Infect Dis* 1997; 24: 64-74.

Golub B, Falk G, Spink WW. Lung abscess due to *Coryneform equi*. Report of first human infection. *Ann Intern Med* 1967; 66: 1174-7.

Harvey RL, Sunstrum JC. *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. *Rev Infect Dis* 1991; 13: 139-45.

Hondalus MK, Mosser DM. Survival and replication of *Rhodococcus equi* in macrophages. *Infect Immun* 1994; 62: 4167-75.

Kanaly ST, Hines SA, Palmer GH. Transfer of a CD4+ Th 1 cell line to nude mice effects clearance of *Rhodococcus equi* from the lung. *Infect Immun* 1996; 64: 1126-32.

Linder R. *Rhodococcus equi* and *Arcanobacterium haemolyticum*: two "Coryneform" bacteria increasingly recognized as agents of human infection. *Emerg Infect Dis* 1997; 3: 145-53.

Mayor B, Jolidon RM, Wicky S, *et al*. Radiologic findings in two AIDS patients with *Rhodoccocus equi* pneumonia. *J Thorac Imaging* 1995; 10: 121-5.

Muntaner L, Leyes M, Payeras A, *et al*. Radiological features of *Rhodococcus equi* pneumonia in AIDS. *Eur J Radiol* 1997; 24: 66-70.

Prescott JF. *Rhodococcus equi*: an animal and human pathogen. *Clin Microbiol Rev* 1991; 4: 20-30.

Scott MA, Graham BS, Verrall R, Dixon R, Schaffner W, Tham KT. *Rhodococcus equi* - an increasingly recognized opportunistic pathogen. Report of 12 cases and review of 65 cases in the literature. *Am J Clin Pathol* 1995; 102: 649-55.

Van Etta LL, Filice GA, Ferguson RM, *et al*. *Corynebacterium equi*: a review of 12 cases of human infection. *Rev Infect Dis* 1983; 5: 1012-8.

Verville TD, Huycke MM, Greenfield RA, Fine DP, Kuhls TL, Slater LN. *Rhodococcus equi* infections of humans. 12 cases and a review of the literature. *Medicine* 1994; 73: 119-32.

Zink MC, Yager JA, Prescott JF, Fernando MA. Electron microscopic investigation of intracellular events after ingestion of *Rhodococcus equi* by foal alveolar macrophages. *Vet Microbiol* 1987; 14: 295-305.