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Corynebacterium pseudotuberculosis infection in equines, Tirupati, Andhra Pradesh

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Abstract
Skin infection with abscess formation was observed on thigh regions of equines. Pus swabs from abscess were collected for diagnosis. Pus swabs were inoculated into nutrient broth and then streaked on to the blood agar plates for cultural isolation. Small, dry white colonies were observed after 24hrs of incubation initially. Later creamy white haemolytic colonies with a narrow zone of haemolysis were observed after 72hrs suggestive of *Corynebacterium pseudotuberculosis*. Further, the isolates were confirmed biochemically showing positivity to catalase, methyl red and urease tests and negative for oxidase, indole, voges proskers and nitrate reduction tests. Direct microscopic examination of cultural smears revealed gram positive pleomorphic rods on grams staining. The antibiotic sensitivity/resistant pattern of the isolates revealed sensitivity to Pencillins, followed by Gentamycin, Kanamycin, Streptomycin and Chloramphenicol and resistance to Tetracylins, Amoxicillin, Ampicillin and Nitrofurans respectively.

Keywords: abscess, equines, dry white colonies, pleomorphic rods, sensitivity and resistant patterns

Introduction
Corynebacterium pseudotuberculosis is a common cause of infection in horses and cattle leads to chronic abscesses on the limbs and abdomen and ulcerative lymphangitis. (Aleman *et al*, 1996) Corynebacterium pseudotuberculosis is a gram positive pleomorphic facultative intracellular bacterium (Brown and olandar, 1987) [5]. *C pseudotuberculosis* can be classified into two biovars, based on their ability to convert nitrate to nitrites, nitrate positive strains are classified as biovar equi and the nitrate negative ones as biovar ovis (Biberstein *et al*, 1971) [3]. In sheep and goats infection is caused by biovar ovis, whereas horses and buffaloes are mostly infected by biovar equi strains (Selim *et al*, 2001; Pratt *et al*, 2003 and Baird *et al*, 2007) [13, 9, 4]. The infection caused *by C.pseudotuberculosis* is distributed worldwide, causing significant disease in horse, sheep and goat herds. (Baird *et al*, 2007; Guimares *et al*, 2011) [4, 7]. Once established in a farm infection is maintained by contamination of the environment with active draining lesions. Contamination occurs through contact with infected animals and consumption of infected food (Dorella *et al*, 2006; Trost *et al*, 2001 & Peel *et al*, 1997) [6, 14, 10]. The main economic losses attributed to *C. pseudotuberculosis* infection include decreased milk production, decreased weight gain, and reduced value of hides due to scarring, the cost of the drugs and labor need to treat the disease (Guimares *et al*, 2011) [7].

Materials and Methods
Sample collection
Three of the equines at NCC unit, CVSc, Tirupati on clinical observation have dermatitis with abscess formation on thigh regions at four quarters of college of Veterinary Science, Tirupati Andhra Pradesh during June, 2019. Pus swabs were collected aseptically from the dermatic abscesses and samples were processed for diagnosis at SLDL, SVVU, Tirupati by subjecting to cultural isolation.

Cultural isolation
The collected pus swabs were inoculated into nutrient broth and incubated for 12 to 18hrs. Later streaked on blood agar media for colony morphology.

Direct Microscopic examination
Cultural smears were stained with routine grams staining method and observed for morphological characterization of the bacteria.

Bio-chemical tests

Cultural isolates were confirmed by subjection to the IMVIC tests, Catalase test, oxidase, urease, nitrate reduction tests according to Quinn *et al* (1944) [11].

Antibiotic sensitivity test

Antibiotic sensitivity/resistant pattern were studied using disc diffusion method (Bauer *et al*, 1966) [2].

Results and Discussion

Upon cultural isolation, small, white dry colonies were observed on blood agar media after 24hrs of incubation. After 72hrs of incubation white creamy haemolytic colonies with narrow zone of haemolysis was observed suggestive of *C.pseudotuberculosis* (Fig. 1). Cultural smears on grams staining revealed pleomorphic gram positive rods indicative of *C pseudotubersulosis* (Fig. 2). The recovered isolates were further confirmed by bio-chemical tests. The isolates were positive for catalase, methyl red, citrate tests and negative for oxidase, indole, voges proskraseurs and nitrate reduction tests. These results are in-agreement with earlier reports of Dorella *et al* (2006) & Reshma *et al* (2017) [6, 12].

During the study, antibiotic sensitivity of the recovered isolates of *Corynebacterium pseudotuberculosis* revealed the sensitivity to pencillins followed by Gentamycin, Streptomycin, Kanamycin, and Chloramphenicol). Similar sensitivity pattern was observed earlier by Kathlean *et al* (2000) [8]. However, resistant to Tetracyclins, Amoxicillin, Ampicillin and Nitrofurans was observed in the present study. Whereas sensitivity to Tetracylins, Ceftriaxone was reported by Reshma *et al* (2017) [12]. However, variation in sensitivity and resistance pattern was observed by several workers at different geographical areas. This might be due to indiscriminate usage of antibiotics over a period of time for treatment without antibiotic sensitivity testing.

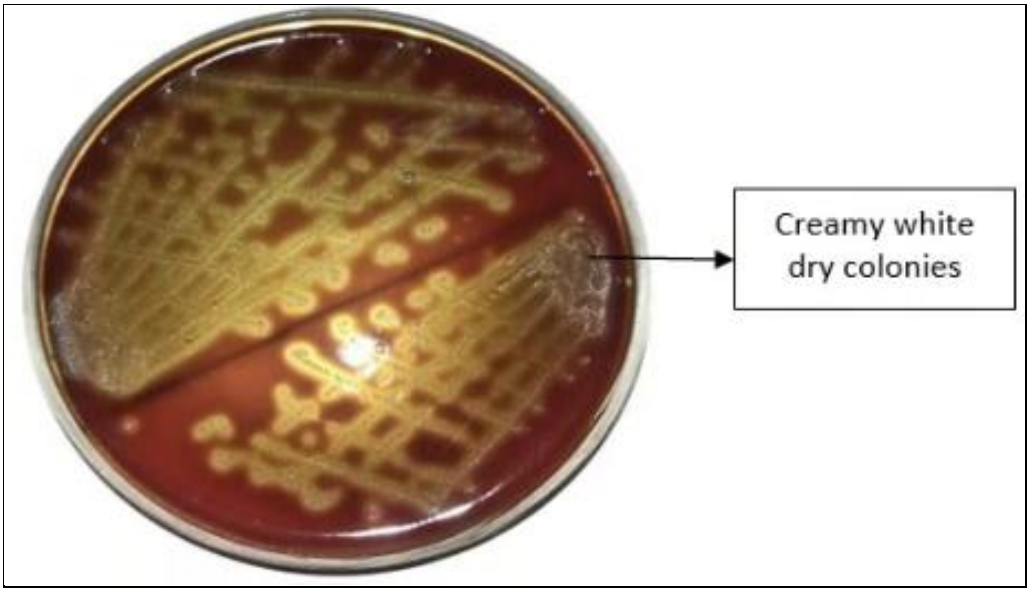

Fig 1: Creamy white dry with narrow zone of haemolytic colonies on blood agar medium

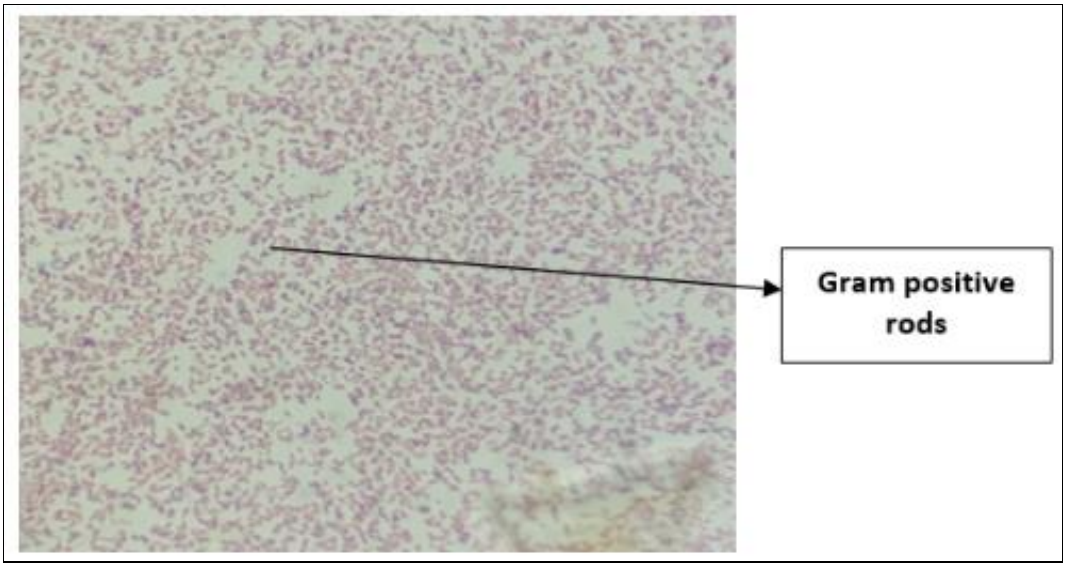

Fig 2: Pleomorphic gram positive rods on Gram's staining

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