

## ISOLATION OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS FROM CAMEL IN IRAN

by

A. H. Esterabadi, F. Entessar, H. Hedayati, A.A. Narimani, M. Sadri

### Introduction

*Corynebacterium pseudotuberculosis* has been known under variety of names. The first description was given by Preiz and Guinard (1891), they found an organism in a renal abscess of a sheep.

Preiz in 1894, named the organism *Bacillus pseudotuberculosis ovis* or Preiz bacillus.

Lehmann and Neumann (1896), introduced the generic name *Corynebacterium* to this entity, as they had not studied Preiz's observations.

In 1923, the American Society of Bacteriologists renamed this organism as *Corynebacterium ovis* in their classifications.

Pseudotuberculosis was dropped as international rules do not allow the use of binominal specific names.

Carne (1939), considered the name *C. ovis* is not fortunate, as the organism occurs in species other than sheep, and in addition, other corynebacteria, but pathogenic and non pathogenic are found on the surface of the skin or in the alimentary canal of this animal. This confusion was cleared up in the 6th Edition of Bergey's Manual (1948), when the present name *Corynebacterium pseudotuberculosis* was adopted.

The purpose of this paper is to record the isolation of organism from Camel by cultural and biochemical tests.

### Materials and Methods

Source of materials. In July 1974, a disease was reported among Camels at Kamal-Abad, 50 Km west of Teheran. Two of these sick animals were submitted to Razi Institute for bacteriological diagnosis. The lesions were charac-

terized by lymphadenitis in the whole of the body. Swabs were taken from pus abscess for bacteriological examinations.

**Cultural characteristics.** Motility testing was prepared by the hanging drops method of a 24 hours old Heart Infusion Broth culture.

Characterization of colonies were studied on Heart Infusion Agar containing 10% bovine serum PH 7.2.

The action of the culture on solidified serum was determined by growth on Loeffler serum. Haemolytic activity was detected by growing cultures in blood agar. The medium was prepared by adding 4 per cent rabbit red cells previously washed three times in normal saline and resuspended to the original volume of the blood to Heart Infusion Agar PH 7.2. The inoculated plates were incubated in airtight jars in a mixture of 10% carbon dioxide and 90% air at 37°C for 24–48 hours.

**Biochemical tests.** Hiss serum water, pH 7.2 was used for the fluid medium. For the fermentation reaction 1 per cent of the carbohydrate to be tested was added to the medium. Andrade's indicator was used to detect acid formation. Readings were made after 1,2,3,5,7,10,15,20, and 30 days.

Litmus milk was used to demonstrate the action of the organism on milk.

Gelatin liquefaction was studied by growing the culture in gelatin, incubated at 37°C and observed every day for 10 days. They were placed in a refrigerator for one hour, each day in order to detect liquefaction.

Hydrogen sulfide production was ascertained by growing on Triple Sugar Iron Agar.

Voges-Proskauer and Methyl-red reaction were determined by growing cultures in VP-MR medium. Nitrite formation by growing the cultures on nitrate agar (Difco) using the test given for this medium and indole formation by growing the cultures in tryptophane broth.

**Animal virulence.** Rabbits and Guinea pigs inoculated intracutaneously and intraperitoneally with 0.1 and 0.2 ml of the 72 hours old cultures suspension respectively. Animals were examined for gross pathology and the heart's blood was cultured on selective media.

## **Results**

**Morphological.** Cultural and Biochemical investigations: According to the microscopic picture, the strain consisted of short and coccobasillus. Diameter varies between 0.3 to 1.0  $\mu$ . The organism is strongly gram positive, non motile, has a granular appearance when stained with Neisser's method (Fig 1).

The strain grew rapidly after overnight incubation at 37°C on 10% bovine serum agar. Colonies were rather small though their diameters enlarged up to 1.0 after a few days.

Growth was not improved by incubation in a CO<sub>2</sub> atmosphere and a comparatively good growth was obtained in aerobic conditions. The effect on rabbit blood agar after 48 hours incubation showed haemolysis in the depth of the medium (Fig 2).

After growth for two days in Heart Infusion Broth, the medium was moderately turbid and a flaky deposit was noticed, a thin pellicle on the surface, with a tendency to rise up along the wall of the tube was noted. Growth in peptone media, including the MR-VP medium was negative. Growth occurred in Triple Sugar Iron Agar slants with no change at the butt.

Acid was produced from glucose, mannose, fructose and maltose. Nitrogen gas was not detected with inverted Durham tubes. Urea was hydrolysed and indole was negative. Liquefaction of gelatin and coagulated bovine serum gave negative results.

Animal virulence. Intracutaneous injection of organism produce in rabbits a characteristic elevated, plateau like abscess. A zone of redness and edema appeared in approximately 6 hours and followed by central pallor and necrosis at 24 hours. The lesion reached its maximum size between 24 and 48 hours, when central zone of necrosis and suppuration measured from 5-12mm in diameter (Fig 3).

Intraperitoneal injection of bacterial suspension killed Guinea pigs 48 hours later. The only lesions at necropsy were an early focal haemorrhagic pneumonia and cloudy swelling of the viscera. The organism were demonstrated in the heart blood. There were no change in the adrenals.

## Discussion

The organism causes a condition known as caseous lymphadenitis or "cheesy gland disease.

The first report of infection in sheep was given by Preiz & Guinard (1891). Subsequently cases of a suppurative lesion in sheep, goats, cattle and horses taking the form of a caseous lymphadenitis were reported in different parts of the world by Carre & Bigoteau 1908, Sivori 1899, Cherry and Bull 1899, Gilruth 1902, Norgaard and Mohler 1899, Nocard 1896, Hall and Fisher 1915.

Carpano 1932, described a disease of Camels in Egypt and Asia.

Caseous lymphadenitis has not, in the past been recognized in camels in Iran. Isolation of organism from pus abscess proved to be due to endemic

infection with this organism. The above finding emphasized the need of an investigation on the occurrence of the lymphadenitis associated with *Corynebacterium pseudotuberculosis* among Iranian Camels.

### **Summary**

*Corynebacterium pseudotuberculosis* strain has been isolated from Camel in Iran. The strain was identified on the basis of bacteriological investigations, cultural, and biochemical tests and also pathogenicity for animal laboratory.

### **Acknowledgements**

The authors are thankful to Dr. M. Piechaud, from the Pasteur Institute of Paris for confirmation of the isolated strain, also to Miss Sarhaddi for her technical assistance.

### **References**

- Bergey, D.H. (1948) *Manual of Determinative Bacteriology*. 6th edition. Baltimore. Williams & Wilkins.
- Carne, H.R. (1939) *J. Path. Bact.* 49, 313
- Carre, H. & Bigoteau, L. (1908) *Rev. Gen. Med. Vet* 11,369
- Cherry, T. & Bull, R.J. (1899) *Intercolon. Med. J. Aust.* 4,250
- Carpano, M. (1932) *Boll. Sez. Ital. Sci. Int. Microbiol.* 4,108
- Gilruth, J.A. (1902) *J. Comp. Path.* 15,324
- Hall, I.C. & Fisher, C.V. (1915) *J.Amer. Vet. Med. Ass.* 48,18
- Lehmann, K.B. & Neumann, R.O. (1896) *Atlas und Grundris der Bakteriologie und lehrbuch der speziellen bakteriologischen Diagnostik*. Munich.
- Norgaard, V.A. & Mohler, J.R. (1899) *U.S.Dep. Agric. 16th Ann. Report. Bur. Anim. Ind.*
- Nocard, E. (1896) *Ann. Inst. Pasteur*, 10,609
- Sivori, F. (1899) *Rec. Med. Vet.* 6, 657
- Preiz, H. & Guinard, L. (1891) *J. Med. Vet.* 16, 563

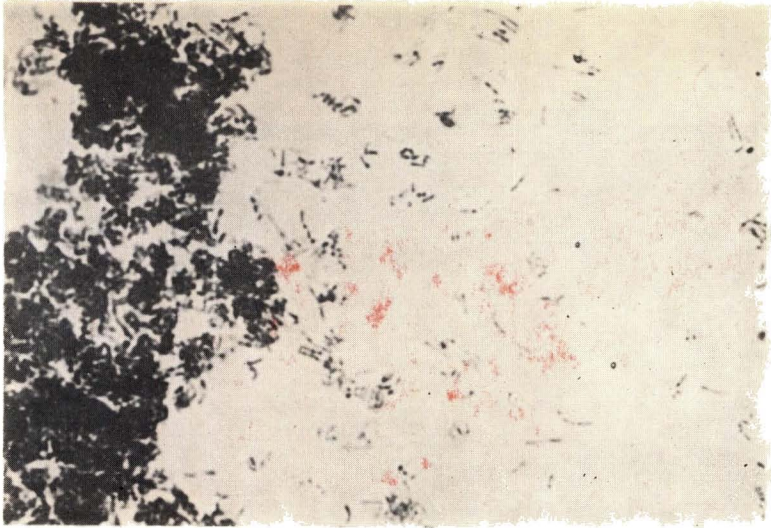


Fig: 1 Granular appearance of *C. pseudotuberculosis* Neisser stain X 1000

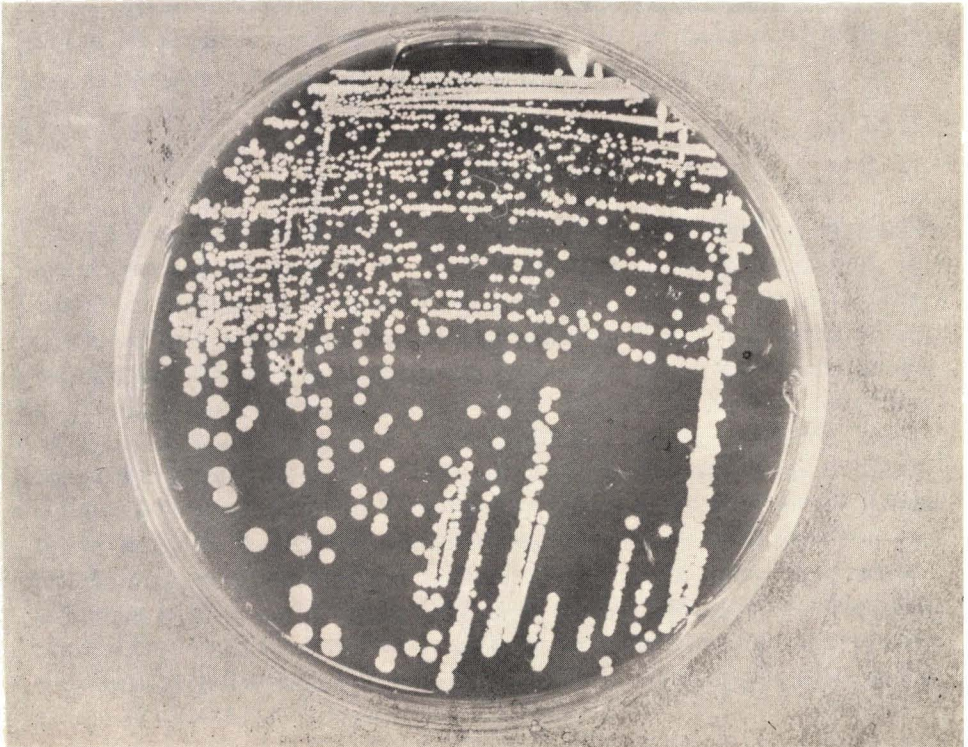


Fig: 2 Colonies of *C. pseudotuberculosis* on blood agar

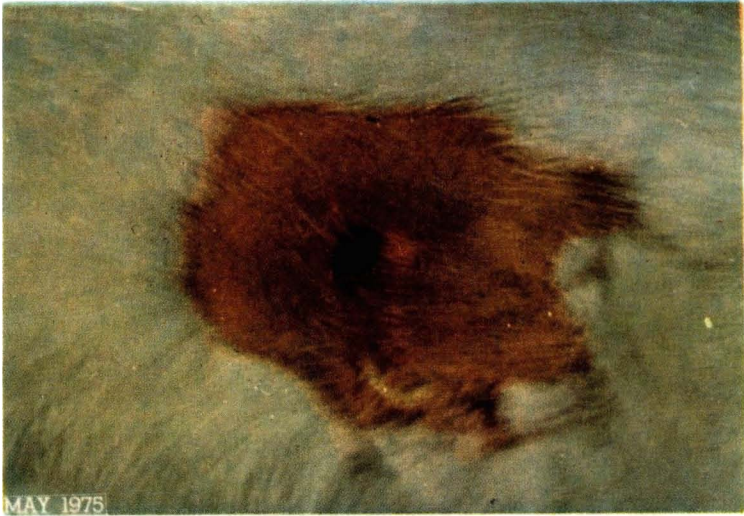


Fig: 3 Necrotizing lesions in skin of rabbit