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Corynebacterium haemolyticum infections in Sri Lanka

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SUMMARY

Corynebacterium haemolyticum infections are described for the first time in Sri Lanka. In a period of 2 years from 1978–80 *C. haemolyticum* was isolated from the pharynx of 9 patients with tonsillitis and from local septic lesions in 7 other patients. Association with other pathogens was common. No patients had a rash. The properties of the isolates are described.

INTRODUCTION

Corynebacterium haemolyticum was first identified as a cause of upper respiratory tract infection and pyoderma by Maclean, Liebow & Rosenberg (1946), who characterized over 150 strains isolated from American servicemen and islanders from the West and South Pacific. Subsequent reports include those of Gärtner & Knothe (1960) from Germany; Herman (1961), U.S.A.; Patocka *et al.* (1962), Czechoslovakia and Ryan (1972), U.K., who found the organism associated with mainly upper respiratory tract infection.

A common feature has been a rash described as itchy, punctate, maculo-papular, erythematous and centrifugal in distribution by Ryan (1972) and as occasionally scarlatiniform or rubelliform (Fell *et al.* 1977).

Extrapharyngeal and extradermal lesions that have been described include empyema (Chlosta *et al.* 1970); cerebral abscess (Washington, Martin & Speikerman, 1971; Altman & Bogokovsky, 1973); septicaemia (Jobanputra & Swain, 1975) and osteomyelitis (Ceily, 1977). Animal infections have been rare, but Richardson & Smith (1968) and Roberts (1969) record isolates from bovine semen and ovine pneumonia respectively.

Fell *et al.* (1977) in their series from Cambridgeshire, UK, recorded 143 cases of *C. haemolyticum* infection over a period of 7 years. The usual site was the pharynx, but isolates were also obtained from varicose and diabetic ulcers and septic lesions of fingers and toes. These reports indicate that the organism has a widespread distribution and is a not uncommon cause of upper respiratory tract infection, skin sepsis and systemic disease in Europe and the U.S.A.

The initial study of Maclean *et al.* (1946), appears to be the only report of *C. haemolyticum* infection in the tropics. There do not appear to be any reports from South-East Asia or Africa.

This communication deals with 16 cases of *C. haemolyticum* infection from Sri Lanka.

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MATERIALS AND METHODS

During a period of 2 years from February 1978 to April 1980 samples of pus and throat swabs, from patients in government hospitals and private surgeries in Colombo and neighbouring provinces sent for routine examination were examined for *C. haemoyticum*. All media and methods were those of Cowan (1974). The throat swabs were streaked on Downie's blood tellurite agar (BTA) and 5% human blood agar (BA) and pus on these and McConkey agar, incubated aerobically and anaerobically and examined with a hand lens at 24 and 48 h.

Physiological and biochemical characteristics

Suspected colonies of *C. haemolyticum* were subcultured and subjected to the following tests. Gram, methylene blue, Albert's stains, catalase, oxidase, O/F, motility, action on glucose, sucrose, lactose, starch, maltose, salicin, mannitol, urea and gelatin. Also for H_2S production and nitrate reduction. Other pathogens found concurrently were subcultured and indentified according to Cowan (1974).

Antibiotic sensitivity tests

Antibiotic sensitivity tests were performed employing a standard disk diffusion method. Antibiotic disks employed were: penicillin, ampicillin, cloxacillin, cephaloridine, erythromycin, tetracycline, chloramphenicol, sulphonamide, cotrimoxazole, neomycin, streptomycin, gentamicin, fucidin and bacitracin.

Animal pathogenicity

Guinea pigs and rabbits were inoculated intradermally with 0·2 ml of a suspension from a 48 h culture on Loeffler's Serum Slopes and the evolution of the lesion so produced, followed up to the end of one month. No diphtheritic antitoxin was administered previously.

Agglutination tests

In all 16 cases a sample of serum was obtained but in only one instance could paired sera be obtained. The technique for agglutination was modified from Jobanputra (1979, personal communication). A 24-h culture from human blood agar was washed off and re-suspended in 1·5 ml of N saline containing 0·1 formalin and heated at 56 °C for 30 min. The deposit obtained after centrifugation was re-suspended in N saline and adjusted to yield $1{\cdot}5 \times 10^9$ organisms/ml employing Brown's opacity tubes. Some cultures were rough but were made stable by squirting repeatedly through a narrow bore pasteur pipette. 0·1 ml aliquots of dilutions of sera ranging from 1:2–1:1024 were made with N saline in W.H.O. plastic agglutination trays. 0·1 ml of the bacterial suspension was added to each cup and results read after overnight incubation in a moist chamber at 37 °C. Serum and antigen controls were also employed.

RESULTS

16 strains of *C. haemolyticum* were isolated; 9 from the upper respiratory tract and 7 from extra-pharyngeal lesions.

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Upper respiratory tract infections

Nine patients were positive – 3 males and 6 females, of ages from 5–21 years, and 7 of them were under 11 years old. Eight were from within 50 km of Colombo, at an elevation of 50 m, but one was from the hill country about 135 km from Colombo, and elevation 500 m. Seven cases presented as follicular tonsillitis with accompanying pharyngitis and pyrexia which ranged from 37–39·5 °C. Two had bilateral tonsillar lymphadenitis. No accompanying rash was observed. *C. diphtheriae* was isolated simultaneously in 5 cases, *Streptococcus pyogenes* in 3 cases, *Staphylococcus aureus* and *E. coli* in 2 cases, *Ps. aeruginosa* in one case. It is noteworthy that *C. haemolyticum*, *C. diphtheriae* and *Streptococcus pyogenes* occurred in 2 cases and *C. haemolyticum* and *C. diphtheriae* in 4 cases. One case with *C. diphtheriae* showed accompanying tachycardia and extrasystoles.

Extra-pharyngeal infections

There were 7 patients in this group, 3 males and 4 females aged from 5 days to 70 years. All patients were from within a radius of 80 km of Colombo. There were 2 cases of foot ulceration, 1 with cellulitis of the legs, 1 omphalitis, 1 otitis media and 2 cases, site of infection unstated. Rash was not an accompanying feature. *C. haemolyticum* was not isolated from contacts. Haemolytic streptococci – Groups A and C, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found simultaneously in 5 of the 7 cases.

All cases were treated with penicillin or erythromycin for 10 days. The organism was eliminated in all instances but recurred in 2 cases (both pharyngitis) after one month.

Bacteriological examination

24-h aerobic incubation on human blood agar yielded a heavy growth of diphtheroid-like colonies with a narrow zone of beta haemolysis. At 48 h, the colonies were 2–3 mm in diameter with a matt surface, entire edge and a wide zone of complete haemolysis. A characteristic feature by transmitted light was the presence of a black round spot at the centre of each colony with a corresponding dimple on the surface. An opaque spot remained on the medium after removal of the colony (Plate 1). Older colonies showed a black lenticular central zone which was adherent to the underlying medium (Plate 2). The colonies grew equally well in an anaerobic environment but with a wider zone of haemolysis. Growth was not enhanced by CO_2. There was no growth on Downie's blood tellurite agar after 72 h and on repeated subculture. Gram-stained films of 24-h culture showed weakly gram-positive pleomorphic 1–3 μm × 0·5 μm bacilli in clumps. Clubbed and Chinese letter forms suggestive of *C. diphtheriae* were not observed nor did bacilli show pallisading characteristic of diphtheroid bacilli. Early (18 h) cultures however showed abortive branching. Metachromatic granules were seen on Albert stained films of 72 h cultures in every case. All strains grew readily in enriched liquid media such as brain heart infusion and Todd Hewitt broth with a diffuse turbidity and granular deposit. No growth occurred in nutrient broth or agar.

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Table 1. *Properties of* C. haemolyticum

Glucose	A	Sucrose	V
Lactose	A	Gelatin	LL
Maltose	A	Salicin	—
Starch	AL	H_2S	—

Acid = Acid. AL = Acid late. LL = Liquefaction Late. V = Variable. — = No reaction.

Physiological and biochemical properties

All strains were non-motile, catalase and oxidase negative and did not grow in Hugh–Liefson OF medium. Tests for urease, indole and nitrate reduction were negative. No acid was produced in mannitol, xylol or glycerol. Results of other tests are shown in Table 1.

Animal pathogenicity

Intradermal injection into guinea pigs produced a 1 cm diameter necrotic lesion in 2 days. This reached a maximum size of 3 cm at the end of one week which sloughed in 10 days leaving a shallow ulcer. Similar inoculation of rabbits produced at 48 h, a 2 cm diameter pustule surrounded by erythema and induration. Subsequent development of this lesion was characteristic. At 1 week the lesion was necrotic and hour-glass shaped; the upper and lower portions being approximately 6 and 8 cm in diameter respectively. The sloughs separated in 2 weeks leaving contiguous ulcers which healed uneventfully. In all instances, the microbes were isolated in pure culture from the lesions. There was no apparent protection with diphtheria antitoxin.

Agglutination tests

The titres recorded in the single samples of sera ranged from 32 to 256, the values tending to be higher in those taken later in convalescence. The acute and convalescent samples in the one case yielded values of 4 and 1024 respectively.

Sensitivity tests

All strains were sensitive to penicillin, ampicillin, cloxacillin, carbenicillin, erythromycin, gentamicin, tobramycin, chloramphenicol, cephaloridine and fucidin; the majority to streptomycin, bacitracin and tetracycline. All were uniformly resistant to sulphonamides, cotrimoxazole, neomycin and polymyxin.

DISCUSSION

The incidence of pharyngitis and pyoderma were comparable in our series, unlike the earlier studies of Maclean *et al.* (1946) and Fell *et al.* (1977), where the former were much more common. Upper respiratory tract infections as described by Fell *et al.* (1977) was observed in a younger and narrower age group (5–21 years) than was pyoderma (5 days–70 years). Clustering of cases occurred during the hot humid months of the year: March to August (average temperature 32 °C and relative humidity 80). The occurrence of all cases within 100 km of Colombo indicates the non-availability of laboratory facilities in remoter areas of the country rather than the absence of infections.

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Throat symptoms were mild to moderate in intensity, and consisted of follicular tonsillitis infrequently accompanied by cervical adenitis and pyrexia. Septic lesions ranged from multiple ecthymeta and infective eczematoid dermatitis to omphalitis and otitis media. No rash was observed in any case, which is at variance with the experience of authors in temperate lands (Maclean *et al.* 1946; Chlosta *et al.* 1970; Ryan, 1972; and Fell *et al.* 1977). *C. haemolyticum* was not isolated from any of the contacts. All patients made an uneventful recovery with conventional chemotherapy, although two patients relapsed after one month. Similar instances are cited by Fell *et al.* (1977).

A noteworthy feature was that only in 3 cases, 1 of pharyngitis and 2 of pyoderma was *C. haemolyticum* found in pure culture. *C. diphtheriae* and *Strep. pyogenes* were found most frequently in throat and the latter and *Staph. aureus* on the skin. This differs from the Cambridgeshire series, where over 90 % of throat infections and 50 % of septic lesions yielded a pure growth of *C. haemolyticum* but is in accordance with the Pacific series where mixed infection was the rule.

Cultural characteristics were as described by previous workers, inhibition on BTA and an adherent black dot and lenticular centre of the colonies on BA being characteristic. All our strains, however, were exceptional in that Albert stained films at 72 h displayed metachromatic granules. Physiological and biochemical properties too, differed in many respects from those described previously in standard bacteriological reference books, Cowan (1974); Wilson & Miles (1975). They were uniformly catalase and nitratase negative, liquefied gelatin and hydrolysed starch slowly and only 50 % of the strains fermented sucrose. In many respects, therefore, they resembled the Pacific and Cambridgeshire strains. Our strains were confirmed as being *C. haemolyticum* at the National Collection of Type Cultures, Colindale, London, UK.

The antibiotic sensitivity results differed in some respects from earlier observations (Ryan, 1972; Altman & Bogakovsky, 1973; Jobanputra & Swain, 1975; Fell *et al.* 1977); in being resistant to sulphonamides, cotrimoxazole and neomycin, but was in agreement in being sensitive to penicillin, erythromycin, chloramphenicol and fucidic acid.

Earlier studies (Ryan, 1972; Jobanputra & Swain, 1975) have shown that convalescent sera agglutinated homologous, formalized cultures at a titre of 1024 to 1500. Our titres were considerably lower being the order of 2 to 256, perhaps because samples were obtained early in disease. One pair of sera taken 2 weeks apart showed an 8-fold rise in titre. A very characteristic feature, not emphasized by previous workers, was the hour-glass shaped necrotic lesions produced by intradermal injection of cultures into rabbits.

The relationship of *C. haemolyticum* to *C. pyogenes* is uncertain, although Barksdale *et al.* (1957) believe that the former is a mutant of the latter. *C. pyogenes* has been isolated infrequently from septic lesions of farm animals in this country, but no human cases have been reported so far. There is no association with domestic animals in our series. It appears that *C. haemolyticum* (when looked for) is a not uncommon cause of upper respiratory tract infection and pyoderma.

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EXPLANATION OF PLATES

PLATE 1

C. haemolyticum × 80. 24 h on human blood agar. Central opaque dot and its adherence to medium after removal of colony.

PLATE 2

C. haemolyticum × 80. 48 h on human blood agar. Dark lenticular centre of colony.

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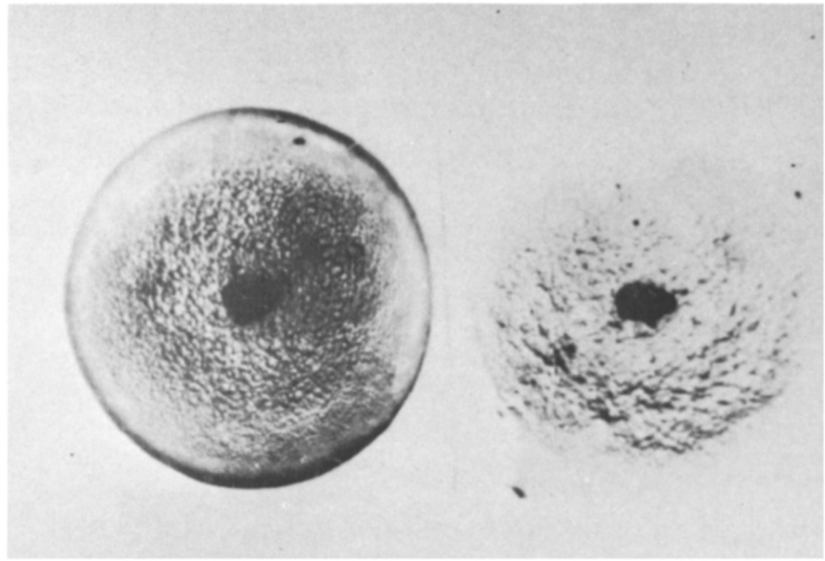

Plate 1

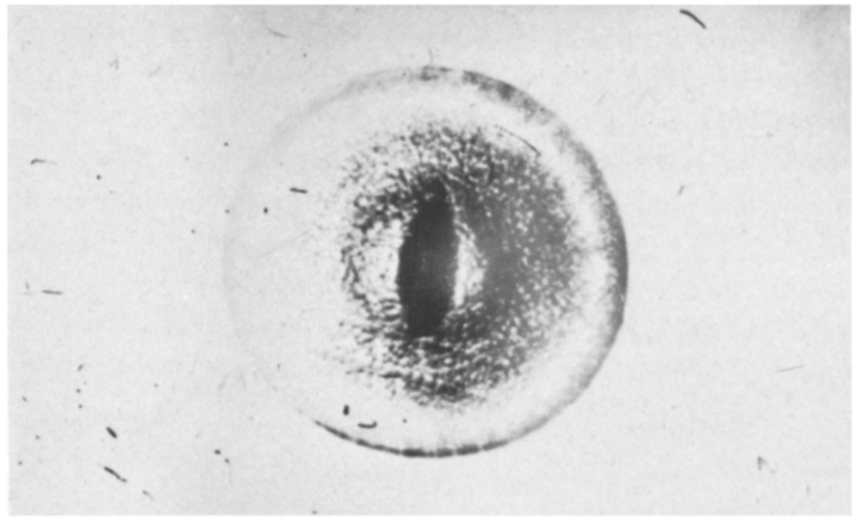

Plate 2

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