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Corynebacterium Equi pneumonia in three Baikal seals (*Pusa sibirica*)

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Abstract

Corynebacterium equi, an opportunistic pathogen of foals, caused necrotic pleuro-pneumonia and lymphadenitis in the three Baikal seals (*Pusa sibirica*) housed in the Antwerp Zoo. Using the selective medium of Woolcock, the organisms were cultured from soil samples collected in outdoor enclosures, but not from the faeces of the healthy animals examined. This result favoured the soil-associated existence of *C. equi*. The isolated strains were tested for their biochemical characteristics and antimicrobial susceptibility.

Introduction

Corynebacterium equi is a saprophytic opportunistic infectious agent affecting animals and occasionally man. The organism was first described by Magnusson[1] in 1923 as an aetiologic agent of foal pneumonia. Since then, infection with *C. equi* has been regarded as an important cause of mortality in young foals all over the world.[2–10] In foals the most important necropsy findings are chronic suppurative bronchopneumonia with abscesses in the lungs and bronchial lymph nodes, ulcerative enteritis and mesenteric lymphadenitis.[8–10] *C. equi* is also frequently isolated from normal or tuberculous submaxillary and cervical lymph nodes in piglets, often in association with avian tubercle bacilli.[11,12] Infection in other animals is rare and has been reviewed by Barton and Hughes.[8]

In recent years there is a tendency to re-classify *C. equi* to the new genus *Rhodococcus* in the family *Nocardiaceae*.[8,13]

The organism is usually regarded as a soil saprophyte[14–17] and can be readily isolated from animal faeces, especially from horses in which it is believed to be part of the normal intestinal flora.[17,18] In the foal, a soil-derived aerosol innoculation is regarded as the most likely route of infection.[10,19]

This article reports on three fatal cases of *C. equi* infection in Baikal seals (*Pusa sibirica*) at the Antwerp Zoo. The clinical and pathological features of the infection, bacteriological characteristics of the isolated strains and the epidemiology are studied.

Case History

The Baikal seals housed in the Antwerp Zoo consisted of a male imported 7 years ago and 2 females who arrived in May 1984. Their enclosure had a rocky waterside and a concrete pool filled with unchlorinated tap water. Apart from a recurring dermatitis, the animals remained in good health during their stay at the zoo. This skin condition was characterised by small, grey-white, nodular cutaneous lesions on the trunk, face and flippers of the animals (Fig. 1). It appeared in spring, coinciding with the moulting-time (March) and disappearing in early summer (June) without any obvious detriment to the animals. Microscopic examination of the cutaneous lesions revealed numerous dermatiaceous hyphae and polymorphonuclear neutrophils, apart from an inconstant and variable microbial flora. *Escherichia coli, Proteus* spp., *Clostridium perfringens* and *Candida albicans* were isolated from the lesions, but were considered as environmental contaminates. Whereas *Alternaria* spp. were repeatedly cultured from scrapings and biopsies of the lesions. Before the latter were taken, the skin was disinfected by scrubbing with 70% ethyl alcohol. As a result, *Alternaria* spp. were isolated in pure culture from inside the lesions. Intestinal parasitism was not diagnosed on preceding examinations.

In September 1985, the animals were placed in quarantine at our facility, as they were suspected to have eaten the fallen autumn leaves in their outdoor enclosure. A few days later, one of the females (Case 1) became listless, refused to eat and had an elevated respiratory rate. On radiographic examination lung consolidation and pleural effusions were obvious. Blood analysis revealed an elevated leucocyte count (21 000/mm^3) with a marked neutrophilia (94%). Diagnosis of pleuro-pneumonia was made and the animal was treated with antibiotics (oxytetracycline 20 mg/kg IM s.i.d.). The animal died within a week.

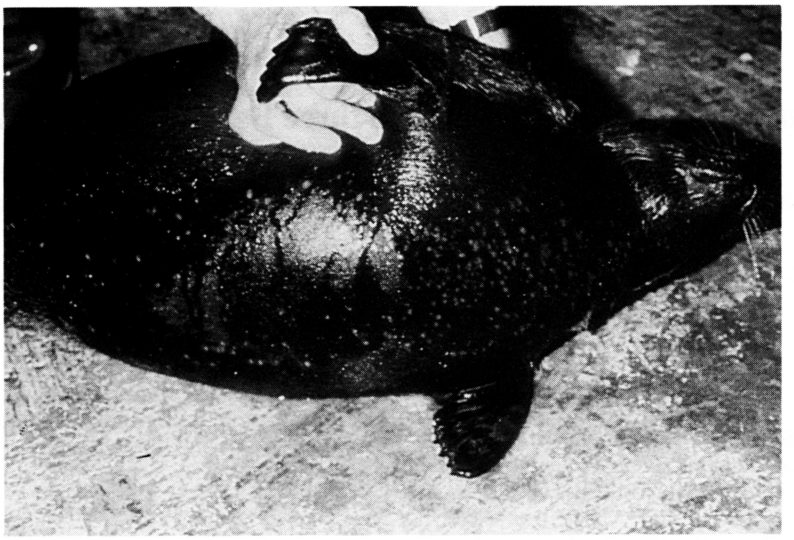

Figure 1. A Baikal seal with cutaneous lesions, from which *Alternaria* spp. were repeatedly cultured.

A month later the male seal (Case 2) became sick presenting the same symptoms and radiological lesions, the animal died after a week despite antibiotic treatment (oxytetracycline 20 mg/kg IM s.i.d.). The remaining female (Case 3) was clinically healthy and received preventive antibiotic treatment for three successive months (trimethoprim 5 mg + sulphamethoxazole 25 mg/kg oral b.i.d.). In February 1986, the animal started to develop the typical skin lesions mentioned above and died after a few days of clinical illness.

Necropsy Findings

In all the three cases the gross pathological findings were very similar. There was thick purulent exudate in the pleural cavities. A nodular lesion, approximately 5 cm in diameter, was diagnosed bilaterally in the anterior part of the lungs (Fig. 2). On incision it was spongy and filled with pus. The bronchial lymph nodes were much enlarged and suppurated (Fig. 2). In all the cases the lymph node in the pancreatic region was also affected. Enlargement of the spleen was noticed in the two last cases. The other organs were normal.

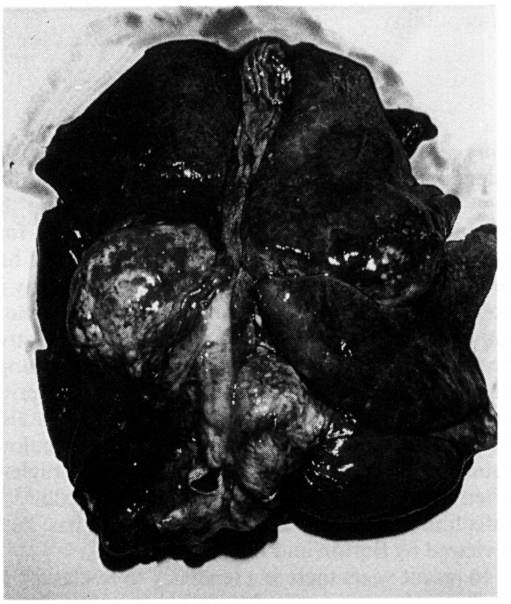

Figure 2. Pulmonary abscesses and a necrotic enlarged bronchial lymph node from a Baikal seal (Case 2) with *Corynebacterium equi* induced pneumonia.

Bacteriologic Examinations

At necropsy, tissue specimens of lungs, pulmonary abscesses, lymph nodes and pleural exudate samples, together with liver and spleen of the last two cases, were collected for bacteriological staining and culture procedures. Gram-stained smears of the lesions showed numerous Gram-positive pleomorphic rods, filling neutrophils and macrophages (Fig. 3). Ziehl-

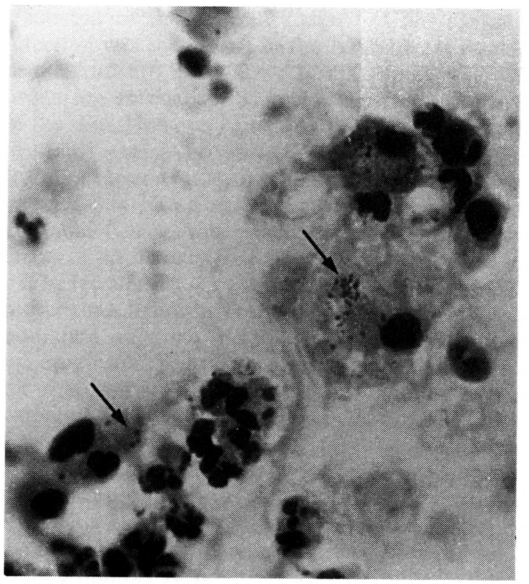

Figure 3. Photomicrograph of purulent discharge from the lung of a Baikal seal, showing *Corynebacterium equi* lying intracellular in neutrophils and macrophages (arrows). Gram's stain. × 1000.

Neelsen staining failed to demonstrate acid fast bacilli. Aerobic cultures of the collected samples were made on blood agar (Casein-peptone Soyameal-peptone agar MERCK) and MacConkey agar (MERCK) plates. Anaerobic cultures were made in thioglycolate broth (MERCK). In all three cases, blood agar cultures of the affected organs yielded heavy growth of nonhaemolytic, glistening and mucoid colonies, with a tendency to coalesce. Sparse growth of similar colonies was obtained from liver and spleen tissues. Gram-stained smears of overnight cultures showed Gram-positive coccobacillae arranged in palisades or L and V shapes. The organisms were identified as *C. equi* by the biochemical characteristics[8] presented in Table 1. On Casein-peptone Soyameal-peptone agar, formation of a slight pink pigment was noticed after a week's incubation at 36°C.

In view of these findings, faecal samples of the other pinnipeds housed in the Antwerp Zoo (South African fur seals *Arctocephalus pusillus*, California sea lions *Zalophus californianus*, South American sea lions *Otaria byronia*, southern elephant seals *Mirounga leonina* and common seals *Phoca vitulina*) together with faecal samples of the fallow deer *Dama dama dama* and Dybowski's deer *Cervus nippon hortulorum* having their enclosure opposite to the Baikal seal's pool, were plated on NANAT medium. This selective medium has been described by Woolcock *et al.*[17] and consists of Casein-peptone Soyameal-peptone agar 40 g, yeast extract (DIFCO) 1 g, nalidixic acid 20 mg, novobiocin 25 mg, cycloheximide 40 mg and potassium tellurite 50 mg in 1 litre distilled water, enriched with 5% horse blood. Samples were moistened with sterile water and innoculated heavily onto the medium. Incubation followed at 36°C for 48 hrs. On this medium *C. equi* forms the characteristic large mucoid and glistening colonies, with a grey to black shade.

Soil or mud samples collected in the enclosures of the aforementioned animals, together with sand from an empty enclosure nearby the Baikal seal's pool were also plated on this medium. The empty enclosure which was examined housed a square-lipped rhinoceros *Ceratotherium simum cottoni*, which died in August 1985. Soil samples were taken after the enclosure remained empty for 8 months. NANAT medium was also used to culture the faeces of the female seal (Case 3) in quarantine, which died 4 months after the previous cases. Despite antibiotic treatment she continually shed *C. equi* once the treatment was interrupted for a few days. No isolations were obtained from the faecal samples of the other animals examined. However, *C. equi* could be isolated from the mud collected on the bottom of the pool and between the rocks of the former enclosure of the Baikal seals. Mud samples collected in the enclosures of the California sea lions and South African fur seals, lying next to the pool of the Baikal seals, and mud from the South American sea lion's enclosure were positive, as were the sand samples collected in the enclosure of the Dybowski's deer and in the empty enclosure which previously housed the rhinoceros.

As a control, the slime-skin of frozen fishes (sprat and whiting) used for feeding were swabbed and innoculated onto NANAT medium. No isolations were obtained from forty different fishes.

Antimicrobial sensitivity testing of the clinical and environmental isolates, using Neo-Sensitabs (ROSCO), was performed on Mueller Hinton agar (MERCK) by the Kirby and Bauer[20] method. All 10 isolates were resistant to penicillin, ampicillin and cefalotin. Four isolates were resistant to chloramphenicol. Sensitivity was noticed to erythromycin, tetracyclines, neomycin, streptomycin and trimethoprim + sulphamethoxazole.

Discussion

Infections of the respiratory system, caused by a variety of bacteria are frequently diagnosed in captive marine mammals. However, reports of infection by *Corynebacterium* spp. are scarce.[21,22] The clinical symptoms and course of *C. equi* pneumonia were similar to the other bacterial pneumonia of pinnipeds. Animals evidenced signs of respiratory distress and general illness. As the disease manifestations

Table 1. Biochemical characteristics of the isolated *Corynebacterium equi* strains. Comparing 3 clinical isolates with 7 environmental isolates

	Clinical isolates			Environmental isolates		
	+	(+)	−	+	(+)	−
Catalase	3	0	0	7	0	0
Oxidase	0	0	3	0	0	7
Motility						
SIM medium (MERCK)	0	0	3	0	0	7
Urease						
Urea broth base (DIFCO)	0	3	0	1	5	1
MIU medium (BBL)	0	1	2	1	5	1
Urea agar base (MERCK)	3	0	0	7	0	0
Nitrate reduction						
Indole-Nitrite (BBL)	3	0	0	5	0	2
H_2S production						
SIM medium (MERCK)	0	0	3	0	0	7
Pb(II) acetate (MERCK)	0	3	0	0	7	0
Arginine dihydrolysis						
Diatabs (ROSCO)	0	0	3	0	0	7
Gelatinase						
Curix X ray film (AGFA)	0	0	3	0	0	7
Aesculin hydrolysis						
Aesculin broth (MERCK)	0	0	3	0	0	7
Bile aesculin agar (BBL)	1	1	1	4	1	2
Diatabs (ROSCO)	0	3	0	0	7	0
CTA medium (MERCK): Acid from:						
Glucose	0	0	3	0	0	7
Maltose	0	0	3	0	0	7
Mannitol	0	0	3	0	0	7
Sucrose	0	0	3	0	0	7
Xylose	0	0	3	0	0	7

\+ Positive reaction within 24 hrs. Incubation at 36°C.
(+) Positive reaction between 2–7 days incubation.
− No positive reaction after a week incubation.

became prominent it had already progressed to an irreversible stage and antibiotic treatment was of no avail (Case 1 and 2). The 3rd case, which received trimethoprim combined with sulphamethoxazole for weeks, also succumbed to the disease. In horses, successful treatment was accomplished with these chemotherapeutics at a higher dosage, though the use of drugs with better intracellular concentration such as erythromycin in combination with rifampicin is recommended.[23] The lesions observed in the present cases are very similar to *C. equi* pneumonia found in foals[24]; empyaema and purulent bilateral pneumonia with severe lymphadenitis being the main pathological findings. In pinnipeds *C. equi* also appears to have an affinity to lung tissue.

The exact pathogenesis of *C. equi* pneumonia is unknown. In horses, infection rarely occurs in adult animals. Most of the recorded cases are from young animals which still have an immature immune system. Depression of the immune system due to intercurrent diseases or viral respiratory infections, makes the animals more vulnerable.[9,25,26] All the reported cases in humans have been from immunologically compromised individuals.[27–29] The affected Baikal seals were all adults. However, the yearly recurrent skin condition seen in these animals may reflect or it may directly provoke a chronic stress situation lowering the immune response and making the animals vulnerable to bacterial and fungal diseases. The skin lesions we noticed (Fig. 1) were very similar to those seen by others in Baikal seals. But in our case the fungi involved were *Alternaria* spp. instead of *Trichophyton* spp.[22] Saprophytic *Alternaria* spp. are known as one of the causative agents of phaeohyphomycosis in the human compromised host.[30]

Inhalation is accepted as the primary route of infection in *C. equi* pneumonia. There are some reports of the involvement of parasitic infections in the transmission of this organism in horses, through the damage of the gut wall, by migrating parasites initiating pyaemia. This theory is supported by evidence where infection disappears after anthelmintic

treatment of the affected studs.[16,31] No parasitic infection was diagnosed in the Baikal seals housed in the Antwerp Zoo. There is a possibility that the organisms entered the body through the ulcerations of the skin. However, infection through skin lesions in foals only produces local abscesses.[32–34] Intestinal lesions due to haematogenous spread or secondary infection after ingestion were not noticed, although *C. equi* was repeatedly isolated from the faeces of the last case which remained in quarantine.

The recovery of *C. equi* from sand or mud samples rather than from the faeces of the healthy animals examined, supports the soil-associated existence of the organisms. Wilson[35] reported on the stability of the organism in soil, isolating it for 12 months from a patch of lawn innoculated with a broth culture. Others stated that *C. equi* survives dessication and direct sunlight for at least a year.[36] The isolation of *C. equi* from the sand of an enclosure that remained empty for 8 months, evidences the resistant state of the organisms occurring in nature. Although only a limited number of premises were examined, it provides us with evidence that *C. equi* is wide-spread in the zoo as a saprophyte. Further research is necessary to determine the potentiality of faecal transmission of the organism in different animal species housed in the Antwerp Zoo and to correlate the incidence with the soil isolations.

The biochemical reactions of the isolated strains correspond with those obtained by Barton and Hughes[8] in a study of 59 isolates from soil and clinical material. Variations in biochemical behaviour of the isolates were due to different sensitivities of the methods used (e.g. urease, aesculin hydrolysis, H_2S production), which emphasizes the need for the development of reliable reference tests. All of our strains were resistant to penicillin and ampicillin, a feature shared by the clinical isolates, whereas atypical and soil-derived isolates were often considered sensitive to these antibiotics.[8]

It is noteworthy that these are the first isolations of *C. equi* obtained in the Antwerp Zoo. To the best of our knowledge this infection in the pinnipeds has not been reported before.

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