

Novel *Propionibacterium* infection in cattle

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Objective To describe four cases of infection in cattle, from geographically different places, with a presumptive new species of *Propionibacterium*, which causes granulomatous lesions in the head, thorax, abdomen, pelvic area and skin.

Procedure Gross lesions, ranging from 0.5 to 15 cm and detected during routine carcase inspection at the abattoir, were submitted to the laboratory for routine testing in the National Granuloma Submission Program.

The bacterium isolated was identified using morphological characteristics, biochemical reactions, cell wall components, products of fermentation and 16S rRNA gene sequencing.

Results Gross lesions submitted for examination consisted of a fibrous outer capsule enclosing thick yellow pus-like material. A Gram-Glynn stain of the histological sections revealed colonies of Gram-positive, filamentous, branching bacteria. Bacteriological culture, cell wall analysis, biochemical reactions and 16S rRNA sequencing identified the organism as a *Propionibacterium* sp closely related to *P cyclohexanicum* and the *P freudenreichii* cluster.

Conclusion This is the first report of a *Propionibacterium* sp closely related to *P cyclohexanicum* and the *P freudenreichii* cluster associated with extensive granulomatous lesions in cattle in Queensland. Sequencing data are suggestive of a previously undescribed species of the *Propionibacterium* genus.

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In mid 1997, as part of the monitoring for bovine tuberculosis under NGSP, an abnormally high condemnation rate for both whole and part carcasses with granulomatous lesions was observed from a line of cattle at slaughter from one north Queensland property. The granulomas submitted for investigation, contained caseous contents, were both numerous and often large, and were found in many sites and organs. The lesions were unlike those caused by *Actinomyces bovis*, *Rhodococcus equi*, *Mycobacterium bovis* and fungi both in their size, distribution and content. The number of lesions, and their widespread location throughout the carcase, the number of organs affected and the extent of condemnations combined with the lack of any previous record of such a syndrome in north Queensland cattle, initiated the following investigation.

Materials and methods

Case Histories

Case 1— In July 1997, beef cattle, which had passed ante-mortem abattoir inspection, were found to have lesions on the peritoneum and in the tongue, lungs, flank muscle and liver. The lesions ranged from 0.5 to 15 cm and contained yellow semi-viscous pus-like material. Six carcasses were condemned and 16 were severely trimmed from a mob of about 100

3-year-old Brahman cross steers from a beef fattening property in northern Queensland. Samples were submitted to OVL in mid-July under the NGSP. Of sixty spayed heifers from this property inspected at an abattoir in late July 1997, two contained lesions, one on the omentum and one on the dorsal peritoneum.

Case 2— In September 1997, 800 3-year-old steers, from an unrelated fattening property in central Queensland were slaughtered. Forty-six animals of the 800 slaughtered had multiple lesions, 2 to 3 cm across, which were found in similar locations to those in case 1. Additional lesions were identified in the ruminal wall and flank muscles.

Case 3— In May 1998, 190 spayed cows and 20 bulls were sent for slaughter from the breeding property owned by the owner of the case 1 property. One cow was condemned at post-mortem inspection and in excess of 30% of animals had multiple lesions throughout the body. The most frequent locations were the external surface of the rumen and reticulum. The lesions resembled those seen in cattle from the fattening property in case 2.

Case 4— In July 1998, samples from cattle from a breeding property in northwestern Queensland with the same owner as the case 2 property were submitted under the NGSP. About 30% of a mob of 324, 10- to 12-year-old cows had lesions. Seventy percent of the lesions were in breeders, many of which were in calf. The other 30% of lesions were in spayed animals. Lesions had a similar distribution to those in cases 1, 2 and 3 above.

Samples of lesions from both the abattoir cases and the field necropsies were submitted to OVL for histological examination and bacteriological culture. Skin samples from the owner of the property described in case one and environmental samples including soil samples from the yards and swabs from the crush of this property were also submitted for bacteriological culture.

Histology

Samples were processed and stained using Haematoxylin and Eosin and Gram-Glynn.

Bacteriology

Samples were homogenised in nutrient broth and then a sample of the homogenate inoculated onto sheep blood agar and MacConkey agar aerobically and sheep blood agar and anaerobe agar under 5% CO₂ and 95% N₂. All the samples

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CFA	Cellular Fatty Acid Analysis
DAP	Diaminopimelic acid
DFA	Direct Fluorescent Antibody
GLC	Gas Liquid Chromatography
NGSP	National Granuloma Submission Program
OVL	Oonoonba Veterinary Laboratory
PHLS	Public Health Laboratory Service, Cardiff, Wales, United Kingdom.
rRNA	Ribosomal RNA
TGH	Townsville General Hospital
VIAS	Victorian Institute of Animal Science

were incubated at 37°C for a period of up to 14 days. Cultures were examined at 3- to 4-day intervals for evidence of growth. Bacteria isolated on anaerobic culture were identified using biochemical tests and cell wall analysis. No Gram stain was done on the original purulent material.

The isolates were also forwarded to TGH for GLC and to VIAS for further testing including GLC to identify products of fermentation and DFA to exclude the organisms from the genus *Actinomyces*. Following results from these laboratories the isolates were sent to PHLS at the University Hospital of Wales, Heath Park, Cardiff, Wales for further identification including a full biochemical screen and GLC analysis. The results from PHLS were inconclusive so the isolates were also sent to the Federal Laboratories for Health Canada, Winnipeg, Manitoba for investigations which included biochemical profiles, GLC analysis, 16S rRNA sequencing and CFA. The methods for analysis of CFA composition were based on those described by Moore et al¹ and Cummins and Moss;² the latter specifically describes the CFAs of propionibacteria. The Canadian conventional methods including biochemical, products of fermentation and CFAs are cited in brief in Bernard et al.³

The 16S rRNA gene sequencing and interpretation are based on those described by Edwards et al,⁴ producing about 1500 base pairs (almost a full sequence). Sequences were compared with those found in the Genbank database. Closest relative matches from a BLAST search were aligned with the propionibacterium-like strains described here and placed on a phylogenetic tree.

A comprehensive description of phenotypic, chemotaxonomic and genetic characteristics of the bacteria, believed after this study to be a novel taxon group, will be reported separately (K Bernard personal communication).



Figure 1. Animal showing an external lesion (arrow) from which *Propionibacterium* sp was isolated.

Results

Case summary

Between July 1997 and July 1998, 2080 cattle in 15 lots from these four case properties were monitored. Fifteen carcasses were condemned and about 250 were trimmed. The most common sites for lesions were the external surface of the rumen and reticulum, in the tongue and on the peritoneal surface of the gastrointestinal tract. Less common sites included the omentum and the flank area, including the flank muscles. Sites where lesions occurred rarely included the lungs, liver, spleen, adipose tissue, mesenteric and pharyngeal lymph nodes, externally on the skin over the ribs, on the cheek and under the jaw. The lesions ranged in size from 0.5 to 15 cm in diameter. They consisted of a fibrous outer capsule, which enclosed thick, yellow, semi-viscous contents. Gritty or granular material was also present.

All the lesions found until mid-1998 were from slaughtered animals with no external lesions seen on antemortem inspection. In June and October 1998 one animal was necropsied on each of the breeding properties (case 3 and case 4). The animals had multiple external lesions ranging from 0.5 cm to 15 cm (Figure 1) and, on necropsy, multiple internal lesions were also found (Figure 2). External lesions were observed on at least 25 mature cows on the property from case 4. *Propionibacterium* sp was isolated from 13 of the 16 lesions submitted from case 1, 3 of the 3 lesions from case 2, 19 of the 25 lesions from case 3 and 11 of the 23 lesions from case 4.

Since July 1998, three more northern Queensland properties have been identified as having the presumptive new *Propionibacterium* sp infection. These isolates have only been identified using conventional biochemical tests and cell wall analysis. This makes a total of seven infected properties to date.

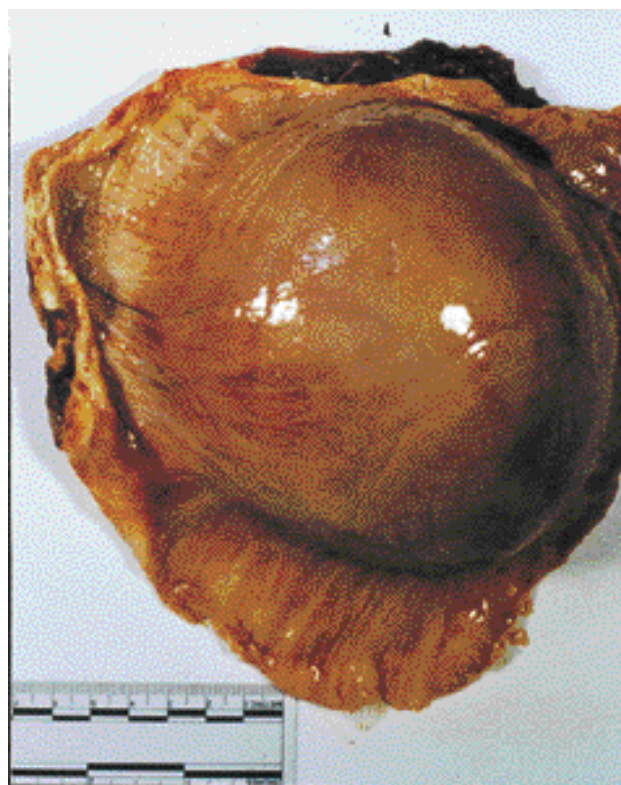


Figure 2. Internal lesion associated with *Propionibacterium* sp.

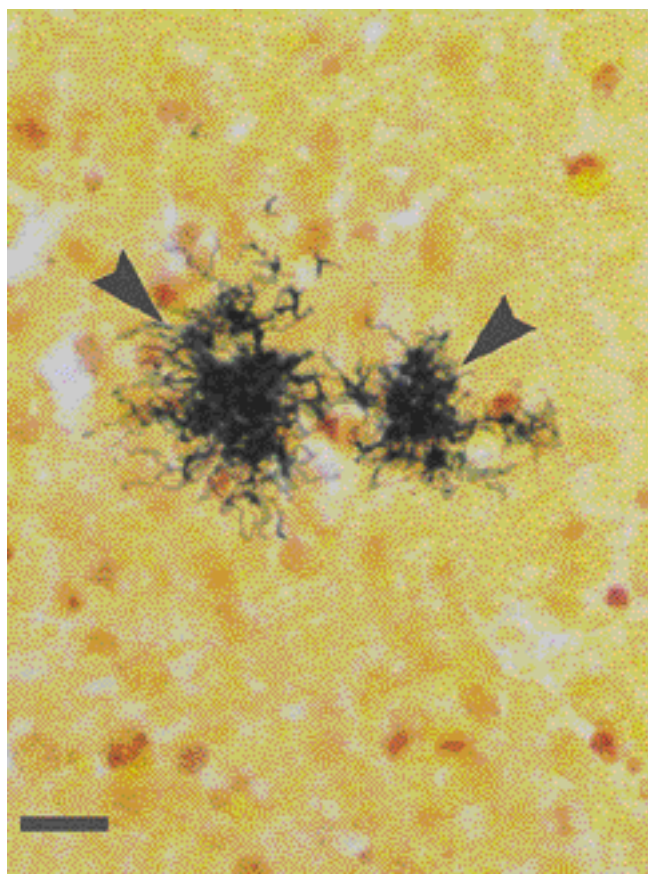


Figure 3. Colonies of *Propionibacterium* sp showing the Gram-positive, filamentous, branching bacteria (arrows) Gram-Glynn stain. Bar equals 10 μ m.

Husbandry practices on these properties include castration, dehorning, botulism and tick fever vaccination, and the use of buffalo fly ear tags and hormone growth promotants on the fattening properties (cases 1 and 2). On the breeding properties (cases 3 and 4) castration, flank spaying, branding, ear marking, dehorning and botulism vaccination are conducted.

Histopathology

Microscopically, the lesions are caseating granulomas and several zones were recognised. An outer zone of fibrous tissue surrounded each lesion. Small foci of lymphoid like cells were sometimes present in this capsular tissue. Adjacent to this, there was a zone of histiocytes and occasional Langhans' giant cells. Next a zone of neutrophils surrounded each bacterial colony. Colonies of bacteria appeared as irregular-shaped masses ranging in size from 18 μ m to 1610 μ m by 920 μ m. These colonies contained a peripheral zone of eosinophilic, hyaline, club-shaped projections with rounded ends. The inner zone of the colonies was frequently pale gray. A Gram-Glynn stain readily demonstrated the organisms as a tangled mass of Gram-positive, filamentous, branching bacteria (Figure 3). In large lesions (Figure 4) the bacterial colonies were sometimes confined to the edge of an amorphous mass of eosinophilic material which contained the scattered nuclei of dead cells. In other instances, the colonies were found throughout the amorphous material.

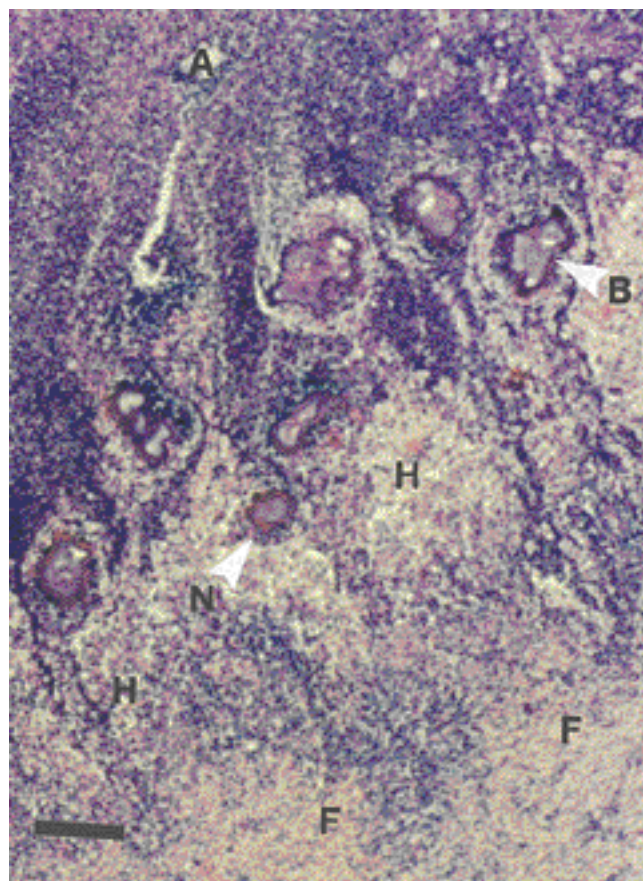


Figure 4. Large lesion associated with *Propionibacterium* sp showing bacterial colonies (B), neutrophils (N), histiocytes (H), amorphous material including nuclei of dead cells (A) and a fibrous capsule (F). Haematoxylin and Eosin stain. Bar equals 180 μ m.

Bacteriology

Creamy, smooth, convex, opaque, round, non-haemolytic colonies were observed after 10 to 14 days of anaerobic incubation at 37°C. The colonies consisted of Gram-positive asporogenic bacilli, which were sometimes short, curved or branched. No significant aerobic bacteria were isolated.

Biochemically, all isolates were positive for aesculin hydrolysis, nitrate reduction, glucose and fructose fermentation and negative for catalase production, indole reaction, maltose and sucrose fermentation.

Propionic acid was observed to be the major volatile acid product of fermentation after analysis at TGH and VIAS using GLC. Thin layer chromatography at OVL revealed the cell walls of the isolates contained meso-DAP. Health Canada performed CFA composition analysis and profiles obtained were consistent with those described for *Propionibacterium* species² with the majority of the CFA's being of the branched chain type.³ DFA tests performed by VIAS for the detection and identification of *Actinomyces israelii* and *A. naeslundii* were negative.

Using 16S rRNA gene sequence analysis, the Australian strains were essentially identical (about 99.9% identity) to each other but had only 91% to 95.5% identity with all other valid *Propionibacterium* species (*P. acnes*, *P. propionicus*, *P. avidum*, *P. granulosum*, *P. acidipropionici*, *P. thoenii*, *P. freundenreichii* cluster, *P. cyclohexanicum*, *P. lymphophilum*).

Discussion

Biochemically, chemotaxonomically and by all genetic identification methods, these pathogens were consistent with, but distinct from, all currently described species in the genus *Propionibacterium*. Microbiological characteristics will be described in greater detail in a separate report (K Bernard personal communication). As presented here in brief, these bacteria were fermentative, anaerobic, asporogenic Gram-positive bacilli which produced propionic acid as the major product of fermentation, had a CFA composition profile consistent with members of that genus and were by 16S sequence analysis, most closely related to *Propionibacterium* species. The PHLS had also reported that the biochemical reactions and the products of fermentation were consistent with those described for propionibacteria. *P. propionicus* (formerly named *Arachnia propionica*) is the only catalase negative organism to date reported to cause actinomycosis in cattle⁵ and has L-DAP in its cell wall.⁶ The isolates reported here somewhat resembled that species biochemically. However they differed by being able to hydrolyse aesculin, were reaction negative for maltose and sucrose fermentation and had meso-DAP rather than L-DAP identified as a cell wall component. Members of the *P. freundenreichii* cluster are aesculin positive and contain meso-DAP in their cell walls, but differ from the new taxo group as being catalase positive.⁶

Propionibacteria have traditionally been grouped according to habitat. The organisms isolated from cheese, dairy products and plants are referred to as the classical propionibacteria and the strains found on human skin, in the mouth and in the intestine as the acnes group or cutaneous propionibacteria.⁷ Organisms belonging to this second grouping are those responsible for infections in animals and humans. The presumptive new species described here differs from this grouping as it is more closely related to the classical or dairy *P. freundenreichii* cluster.

The separation of these two groups is also evident by examining their cell wall composition. The dairy group of propionibacteria have meso-DAP while the cutaneous group has L-DAP or no DAP in their cell walls. In contrast to this the presumptive new species, while causing abscess-like lesions in cattle similar to those lesions caused by *P. propionicum*, *P. granulosum* and *P. avidum* in humans, contains meso-DAP in its cell wall.

Organisms in the cutaneous group including *P. acnes*, *P. avidum*, *P. granulosum* and *P. propionicum*, have been implicated in human infections including cervico-facial actinomycosis, lacrimal canaliculitis,⁵ oligoarthritis associated with pustulosis and acne⁸ and a splenic abscess as a result of cardiac catheterisation.⁹ In all these cases external lesions such as acne and pustulosis or direct introduction, by evasion of the body's defences, allowed the infection to become established.

In the case of *P. propionicum* infection in cattle¹⁰ the lesions resembled actinomycosis caused by *Actinomyces bovis*.

Preliminary investigations into the source of infections by the presumptive new *Propionibacterium* sp concentrated on the possibility that the organism was spread by invasive procedures such as castration or spaying, which occur on these properties. However the finding of lesions in both entire and castrated or spayed animals negated this theory as the sole method of infection. However it is possible that vaccination or other surgical procedures may have contributed to the spread of infection to susceptible animals.

There is a possibility that this organism is present in small numbers on the skin of the animals, similar to *P. acnes*, and infects animals during licking or abrasion caused by close contact with the walls of the crush. This theory has been put forward after finding external lesions on cattle in case 4, indicating a possible spread of infection from animal to animal by direct contact through licking, or by indirect contact via the crush walls during husbandry procedures.

The failure to isolate the organism from the owner or environmental samples from the property in case 1 indicates the most likely method of infection is animal to animal, possibly via transfer through invasive procedures. However it is also possible that the techniques used to attempt isolations from the skin of the owner and environmental samples were inadequate as propionibacteria are constituents of the normal flora of the skin and soil. These problems may have been exacerbated by the transport conditions, as the property is a considerable distance from the laboratory. Further environmental sampling is proposed to attempt to overcome this deficiency.

Further work is proceeding to investigate the extent of these infections in cattle and the possible predisposing causes and routes of infection.

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