Australian
VETERINARY
JOURNAL

SCIENTIFIC SECTION

EDITOR
COLIN WILKS

ASSOCIATE EDITOR
KEITH HUGHES,

EDITORIAL COMMITTEE
NORMAN ANDERSON, GLENN BROWNING, COLIN CHAPMAN, ROBIN CONDRON, JOHN DUFTY, TREVOR FARAGHER, KEITH HUGHES, ALAN LAWTHER, CARL PETERSON, ANDREW TURNER, COLIN WILKS

EDITORIAL ASSISTANT AND DESKTOP PUBLISHING
ANNA GALLO

The Australian Veterinary Journal welcomes original contributions on all aspects of veterinary science.

Submission of a manuscript for publication will be held to imply that it is a record of unpublished original work of the author(s) and, if accepted will not be published elsewhere in whole or part without permission. The Journal reserves the right to reject any manuscript.

Send contributions to the Scientific Section of the AVJ to:
Editor,
AVA House, 272 Brunswick Road,
Brunswick, Victoria 3056.

Telephone: (03) 9387 2982, 9387 8808.
International -61 3 9387 8808
Telefax: (03) 9388 0112,
International +61 3 9388 0112.
Email avjsc@ava.com.au.

'Instructions for authors' were published in the February 1999 issue. They can be found in http://www.farmwide.com.au/nff/vetasscn/aboutava/instruct.htm.

'Statistical guidelines for authors' were published in September 2000. They can be found in http://www.farmwide.com.au/nff/vetasscn/aboutava/statguid.htm.

Mastitis, polyarthritis and abortion caused by *Mycoplasma* species bovine group 7 in dairy cattle

S HUM[a], A KESSELL[b], S DJORDJEVIC[a], R RHEINBERGER[b], M HORNITZKY[a], W FORBES[a] and J GONSALVES[a]

Objective To report an outbreak of mastitis, polyarthritis and abortion caused by *Mycoplasma* sp bovine group 7 in three large, centrally-managed dairies and to review the relevant literature.

Design Epidemiological, clinical and laboratory data were analysed, collated and reported. Multidisciplinary procedures were employed. These included clinical assessment and comprehensive laboratory investigations of affected calves, aborted foetuses and milk samples. Mycoplasma cultures and genetic analyses of isolates were undertaken to identify the aetiological agent.

Results About 30% of 240 calves usually kept in a calf rearing facility developed severe polyarthritis as a result of *Mycoplasma* sp bovine group 7 infection between 2 and 3 weeks of age. Multiple abortions occurred on these farms. *Mycoplasma* sp bovine group 7 was recovered from the fibrinopurulent synovial exudates of four 14-day-old calves, from the stomach contents and lungs of two aborted foetuses, from 14 of 21 bulk milk and four of 10 mastitic quarters. Three bulk colostrum samples cultured during the outbreak were negative for mycoplasma.

Conclusion *Mycoplasma* sp bovine group 7 caused significant economic losses as a result of polyarthritis, abortion and mastitis. The disease probably originated from udder infections with spread being facilitated by the decreased use of tetracycline in the treatment of mastitis. Neonatal calves were most likely infected by the consumption of milk contaminated with the organism. Abortions presumably resulted from mycoplasmaemia. This appears to be the first report in Australia of bovine abortion resulting from *Mycoplasma* sp infection.

Aust Vet J 2000;78:744-750
Key words: Cattle, *Mycoplasma* sp bovine group 7, polyarthritis, abortion, mastitis.

ELISA	Enzyme linked immunosorbent assay
FSC	Foetal stomach content
GDPT	Gel diffusion precipitin test
MZN	Modified Ziehl-Neelsen stain
PACE	Pestivirus antigen capture ELISA
PCR	Polymerase chain reaction

Mycoplasma sp bovine group 7 is currently an unassigned mycoplasma represented by the type strain PG50.[1] This strain was initially isolated from arthritic calves in south Queensland, Australia and its pathogenicity established by experimental infection.[1] Subsequently, *Mycoplasma* sp bovine group 7 has been reported infrequently as a cause of polyarthritis in calves and mastitis in cows. It has also been isolated from pneumonic bovine lungs and lymph nodes in Australia.[2-6]

Mycoplasmas have been implicated in bovine genital disease since their first isolation from the bovine reproductive tract in 1947.[7] Mycoplasmaemia, with subsequent transplacental infection and abortion, due to *Mycoplasma mycoides* infection is well documented.[8] *Mycoplasma bovigenitalium* and *Mycoplasma bovis* are infrequent causes of bovine abortion.[9-12] Other mycoplasmas implicated in bovine abortion have been reported in North America and include a strain designated as NADL 1 isolated from the abomasal contents of an aborted foetus,[13] a strain identified as 3305 isolated from an 8-

[a]NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Private Mail Bag 8, Camden, New South Wales 2570
E-mail steven.hum@agric.nsw.gov.au
[b]University of Sydney Veterinary Clinic, Camden, New South Wales 2570

month-old aborted foetus[10] and a *Mycoplasma* sp bovine group 7 isolated from tissues from an aborted foetus.[9] Abortion as a result of mycoplasmal infection has not been reported in Australia.

This paper presents a detailed account of an outbreak of *Mycoplasma* sp bovine group 7 infection in three large, centrally-managed dairies, causing severe polyarthritis in calves, mastitis and abortion. At the same time the Australian literature relevant to *Mycoplasma* sp bovine group 7 infections is briefly reviewed.

Materials and methods

Farm management

The three dairies involved in the outbreak were centrally managed and located near Sydney, New South Wales. Farms 1, 2 and 3 contained 1050, 1100 and 850 Friesian cows, respectively. Calf rearing practices on the three farms were similar. Calves were removed from the cows and fed 4 L of top quality colostrum during the first 24 h of life. Each day for the next 2 days they received 4 L of pooled colostrum. At 3 days of age calves from farms 2 and 3 were transported to the central calf rearing unit on farm 1, where usually about 240 calves were housed in individual pens. From day 4, calves were fed 2 L of milk night and morning, and given access to commercial calf pellets. The milk was a mixture of mastitic milk and commercial milk replacer. Calves were kept in the rearing facility for 4 to 6 weeks before being moved to a clean paddock.

Serology

The *Leptospira* Microscopic Agglutination Test (MAT) was conducted on foetal fluids as described by Chappel (1993).[14] Radial immunodiffusion (RID), specific for bovine IgG was used to quantify immunoglobulin G in foetal pleural fluids. Foetal pleural fluids that had detectable levels of immunoglobulin G were tested for antibodies to viruses belonging to the Simbu group using a competitive ELISA, with Simbu group-reactive monoclonal antibodies against Akabane, Aino, Peaton, Douglas, Tinaroo, Thirmi and Facey's Paddock viruses (PD Kirkland unpublished). The pestivirus GDPT and PACE were conducted on foetal fluids and foetal spleen respectively as described by Kirkland and MacKintosh.[15]

Histopathology

Tissues collected for histological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 to 6 μm and stained with hematoxylin and eosin.

Routine bacteriology

Foetal lung, liver and foetal stomach contents (FSC) were cultured directly onto blood agar, MacConkey agar and Skirrow's agar. All plates were incubated at 37ºC. The blood agar plates were incubated from 3 to 7 days in an atmosphere containing 5% CO_2, the MacConkey agar plates in air for 48 h and the Skirrow's agar plates under conditions produced by the Camp*Gen* system (Oxoid Australia, West Heidelberg) for 96 h. Smears of the FSC were examined under dark ground illumination for leptospires and stained by the Gram or MZN method for the demonstration of other bacteria. Joint fluids were processed in a similar fashion to FSCs, except they were not cultured on Skirrow's medium or stained by the MZN method. Other tissues were treated similarly to foetal tissues except Skirrow's agar was not used.

Mycoplasma examinations

Culture – The culture medium used for the isolation of *Mycoplasma* sp bovine group 7 directly from joint fluids, tissues and milk samples was a modified Friis broth as described by Hovind-Hougen and Friis,[16] with some differences. The modified broth and agar medium contained horse serum (20%), calf thymus DNA (0.002%) and βNAD (0.009%). Noble agar (1%) was used to prepare mycoplasma agar. Tissue samples were cut into small pieces (1 mm thick) with sterile instruments, added to mycoplasma broth and incubated overnight at 37°C. The following day the broth was filtered (0.45 μm) and re-incubated at 37°C until a colour change was noted (usually within 48 h). A separate aliquot was also used to inoculate a fresh broth. Joint fluid and milk samples including colostrum were added directly to mycoplasma-broth. Using a sterile inoculating loop, an aliquot of mycoplasma broth was streaked onto mycoplasma-agar and incubated for 48 h at 37°C in 5% CO_2. *Mycoplasma* sp bovine group 7 colonies typically had a fried-egg appearance. Single colonies were filter-cloned three times.[17]

Growth inhibition – Filter cloned mycoplasma cultures were identified using the growth inhibition test.[18] Tenfold serial dilutions of the broth were applied as a drop onto dry mycoplasma-agar plates and allowed to run to the bottom of the plate. The plates were air dried. Small wells were inserted along the streak lines and filled with a panel of rabbit antisera raised to different *Mycoplasma* sp. The plates were initially incubated overnight at 4°C to enable the antisera to diffuse into the agar and then incubated at 37°C in 5% CO_2 for 24 to 48 h and inspected under a stereo-microscope (magnification 10 to 40X).

PCR – Amplification of LppA gene (previously p67) from *Mycoplasma* sp bovine group 7 was performed essentially as described by Frey at al[19] with the following modification. PCR reactions (50 μL) were performed using a Corbett Research thermocycler (PC-960 or FTS 960) and comprised 20 pmoles of each primer (P67BG7-L and P67BG7-R), 170 μmol/L of dNTPs, 5 μL of 10 X buffer (500 mmol/L KCl, 100 mmol/L Tris-HCl pH 8.3), 1.5 mmol/L $MgCl_2$, 1 unit *Taq* DNA polymerase, 10 to 50 ng template DNA and milliQ water to a final volume of 50 μL. Thermocycling conditions started with a denaturation cycle for 2 min (94°C) followed by 10 cycles of 94°C (30 s), 52°C (30 s) a nd 72°C (90 s) and 25 cycles of 94°C (30 s), 50°C (30 s) and 72°C (2 min). A final cycle (72°C, 3 min) completed the reaction.

Electrophoresis – A sample (15 μL) of the PCR reaction was loaded onto a 1.0% agarose gel, prepared using 0.5 x TBE buffer (TBE: 45 mmol/L Tris-HCl, 45 mmol/L boric acid, 1 mmol/L EDTA, pH 8.9). Gels were electrophoresed for 1.5 h (65V) or until the bromophenol loading dye reached 1 cm from the end of the gel. Gels were documented using an Image Analyser (BioRad) and tiff files were saved to diskette.

Restriction endonuclease analysis – Amplification product from the PCR was digested with the restriction endonuclease *Hin*f I. Digestion reactions comprised 30 μL of PCR product, 5 μL of 10x restriction endonuclease buffer H (Roche Diagnostics), 5 U of restriction endonuclease *Hin*f I and sterile milliQ water to a final volume of 50 μL. Digestions were incubated at 37°C overnight. Samples of digestion products (15 μL) were resolved on a 2% agarose (w/v) gel.

Results

Field investigations

In late October and early November 1998, mild stiffness, lameness and polyarthritis were observed in about 30% of calves

at the central calf rearing unit which usually housed 240 calves. Clinical signs were first noticed at around 13 days of age and their severity gradually increased over the next 2 days. At this time, the carpal and tarsal joints were greatly enlarged due to the accumulation of intra-articular fluid. There were fluctuating swellings in the bursae of fetlocks as well as the carpal and tarsal joints. Despite these abnormalities, the appetite of affected animals usually remained normal.

During the clinical course of the disease, stiffness, lameness and joint swelling increased despite treatments with penicillin/ampicillin and tetracycline. As a result euthanasia was performed for animal welfare considerations on about half of the affected calves. In the remaining calves, clinical abnormalities slowly subsided irrespective of treatment, but some carpal joints remained permanently enlarged.

In mid November, when the morbidity peaked, there were between 31% and 36% of calves clinically affected and from then on until the end of December the morbidity remained at this prevalence. About 120 calves died or were euthanased during the outbreak. At the end of December when *Mycoplasma* infection was first suspected, routine preventive tetracycline treatment was implemented. Newborn calves received daily intramuscular tetracycline treatment (Engemycin 100; Intervet, Australia Pty Ltd) at a dosage of 1 mL/10kg for 3 days and from then on very few clinical cases occurred.

In October and November 1998, the abortion rate was reported to have increased on farm 1. On 22 December 1998, 6-month-old aborted twin male and female foetuses were submitted to the laboratory for necropsy from farm 1. A few weeks later, from the central calf rearing unit, four neonatal calves affected with polyarthritis were submitted to the laboratory for necropsy.

Laboratory investigations

Clinical pathology – synovial fluids aspirated from enlarged joints of four calves were typical of a marked purulent exudate. The synovial fluids were cream coloured and turbid with an average protein content of 53 g/L (range from 46 to 57 g/L) and an average total nucleated cell count of $63x10^9$/L in three calves (range from 43.4 to $82x10^9$/L). The number of cells in the synovial fluid from the fourth calf was too high to be counted by the automated cell counting machine. Dif-Quick stained smears revealed marked purulent inflammation. On average, 77% of all nucleated cells were neutrophils (range, 67 to 83%). Most of the remaining cells were macrophages with a scattering of synoviocytes (Figure 1). Neutrophils in all aspirates were well segmented and slightly degenerate but devoid of toxic changes. Macrophages and synoviocytes were well vacuolated and often necrotic or lytic. Haematological examination of a 10-day-old calf revealed moderate leucocytosis ($21x10^9$/L) and neutrophilia ($15x10^9$/L) with a marked left shift, $3.45x10^9$/L neutrophils being nonsegmented (normal 0 to $-0.24x10^9$/L).

Bacteriology – Routine bacterial culture of three joint fluids collected aseptically from different animals at various intervals before the abortion investigation revealed no significant bacterial growth. Mycoplasma infection was suspected and joint fluid forwarded to the laboratory for specific culture. At the same time primary isolates, obtained from the abomasal contents and foetal tissues collected from the aborted foetuses, were processed at the laboratory and suggested mycoplasmal infection.

Abortions – The male foetus had generalised subcutaneous oedema and excessive volumes of straw-coloured, slightly turbid fluid in body cavities and the hip joints. No gross pathological abnormalities were detected in the female. Immunoglobulin concentrations in the pleural fluids measured by RID were greatly increased in both foetuses measuring 1911 μg/mL and 2367 μg/mL respectively (normal < 80 μg/mL). This clearly indicated in utero antigenic stimulation and the likely involvement of an infectious agent. The MAT performed on the pleural fluid revealed no specific antibodies to *Leptospira interrogans* serovars *pomona* or *hardjo* and there were no specific antibodies to pestivirus using the GDPT or Simbu virus using the ELISA. The Pestivirus antigen capture ELISA revealed no viral antigens in the spleen samples collected from the foetuses. Beta-haemolytic colonies were cultured from the stomach contents, liver, and lung from the male twin foetus and from the lung of the female foetus. These colonies could not be stained using the Gram method and subsequently were identified as *Mycoplasma* sp bovine group 7. These were the only two submissions for which the blood agar plates had been fortuitously incubated for 4 or more days. No other significant bacterial species were cultured from these foetuses. On histological examination, the male foetus had moderate suppurative bronchopneumonia characterised by large numbers of neutrophils in bronchioli and associated alveoli (Figure 2). There was a mild to moderate multifocal, subacute epicarditis and interstitial myocarditis in both foetuses indicated by infiltration of a mixture of neutrophils and mononuclear leucocytes.

Necropsy of neonatal calves – Four 14-day-old calves were necropsied during the outbreak and revealed similar gross and histopathological findings. They were in fair body condition. Although the appendicular skeleton was most severely affected, (Figure 3) nearly all diarthroidal joints examined, including the intervertebral, costo-vertebral and atlanto-occipital articulations had some degree of abnormality. The ventral portion of the atlanto-occipital joint was greatly swollen and protruded into the pharyngeal cavity. Upon sectioning of the ventral joint capsule the wall was found to contain multifocal areas of necrosis and haemorrhage. The atlanto-occipital joint cavity contained yellow, thick, viscous fluid with numerous fibrin clots (Figure 4). The joints of the limbs were enlarged and contained yellow-grey, turbid synovial fluid and large yellow fibrin plaques or flocculant fibrinopurulent material. There was periarticular and peritendinous subcutaneous oedema. The bursae were enlarged, oedematous and contained turbid synovial exudate. The synovial membranes were slightly thickened, congested and presented some villous proliferation. Articular cartilage appeared normal. There was generalised lymphadenopathy but the prescapular, popliteal and retropharyngeal lymph nodes were greatly enlarged, hyperaemic and odematous. One calf had a bilateral hypopyon. Histological examination of the affected articulations revealed a severe, diffuse, subacute arthrosynovitis, tenosynovitis and bursitis. Joint cavities contained necrotic debri and copious fibrinopurulent exudate. The atlanto-occipital and costovertebral articulations had more acute histopathologic changes, including haemorrhages, thrombosis, necrosis of the synovial membrane and heavy fibrinopurulent exudation (Figure 5). Regional lymph nodes were odematous. There was a mild segmental neutrophil infiltration of the spinal meninges in two calves. Routine bacterial culture was negative but *Mycoplasma* sp bovine group 7 was isolated from the synovial fluid from the four calves and the prescapular lymph-node of one calf. A few days later in a separate submission, *Mycoplasma* sp bovine group 7 was isolated from the synovial and pericardial

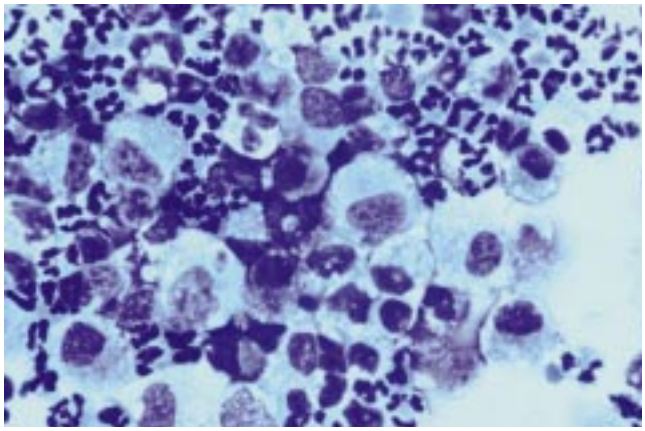

Figure 1. Synovial fluid from the carpal joint of a 14-day-old calf. Marked fibrinopurulent exudate containing neutrophils, macrophages and synoviocytes. Diff-Quik stain (x 900)

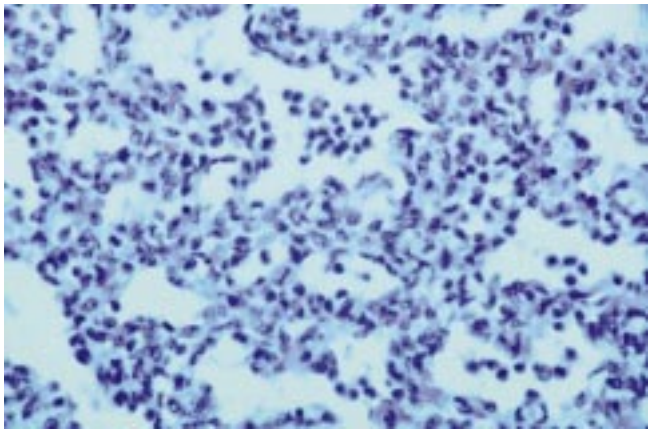

Figure 2. Foetal lung. Suppurative bronchopneumonia characterised by neutrophils in bronchioles and associated alveoli. Haematoxylin and eosin (x 400).

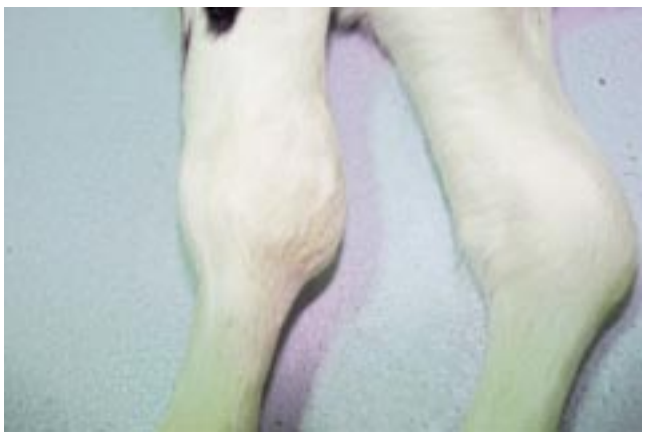

Figure 3. Carpus of a 14-day-old calf. Greatly enlarged diarthroid joints.

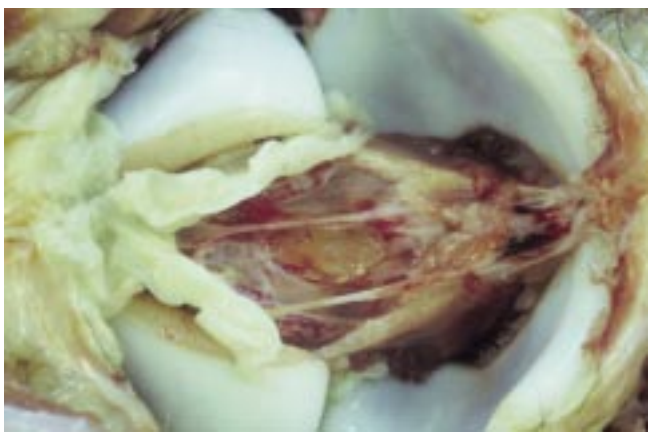

Figure 4. Atlanto-occipital joint of a 14-day-old calf, ventral view. Large yellow fibrin clots in joint cavity, immediately beneath the dura mater.

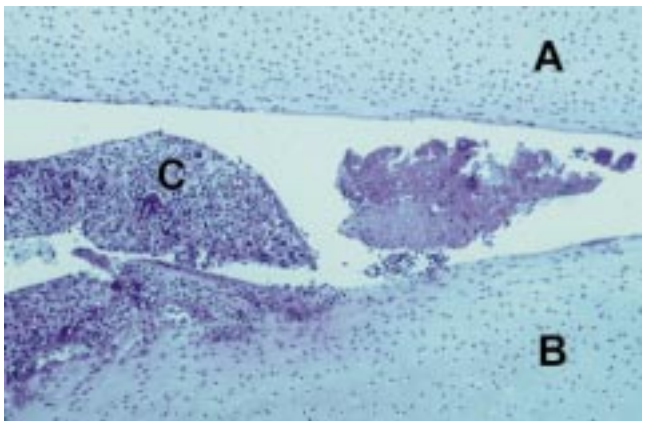

Figure 5. Costo-vertebral joint of a 14-day-old calf. Articular surface of the rib (A), vertebra (B), fibrinopurulent exudate in the lumen (C) and synovial necrosis on the articular surface of the vertebra. Haematoxylin and eosin (x 63).

fluid from one 7-day-old calf necropsied at the central calf rearing unit.

Culture of milk for mycoplasma – During the outbreak 21 bulk milk samples, three samples of bulk colostrum and 10 samples from mastitic quarters were collected at various intervals and cultured for mycoplasma in an attempt to identify and monitor the source of infection. Of the 21 bulk and 10 mastitic quarter milk samples 14 and 4 samples were positive for *Mycoplasma* sp bovine group 7 respectively, but no *Mycoplasma* spp were isolated from samples of bulk colostrum.

Serology and PCR results – Rabbit antisera raised against *Mycoplasma* sp bovine group 7 induced clearing zones in growth inhibition confirming the identity of the isolates. In addition, a fragment of 1.405 kb was amplified when whole cell DNA purified from each of the isolates was used as a template for the *lppA* [Mbgr7] PCR. Digestion of the 1.405 kb amplicon with *Hin*f I generated 5 fragments as described by Frey et al[19] confirming that the 1.405 kb amplicon contained the LppA gene (Figure 6).

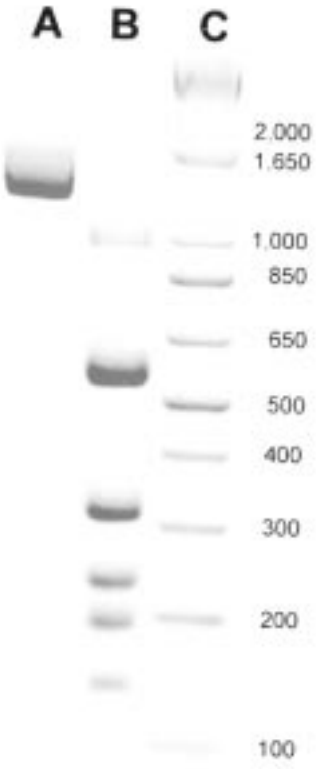

Figure 6. The 1.405 kb PCR fragment containing the LppA gene of *Mycoplasma* sp bovine group 7 (line A) and the products of digestion with *Hin*f I separated by electrophoresis on 2% agarose (line B). The 1 Kb Plus DNA ladder (line C).

Discussion

In the three dairies *Mycoplasma* sp bovine group 7 caused substantial economic losses as a result of mastitis, polyarthritis in calves and possible abortions. About 120 calves died during the outbreak but male calves were sold before the clinical disease was manifest and the total calf losses cannot be estimated accurately. It is possible that the majority of male calves were processed as veal calves in abattoirs while some of them were incubating the disease. Antibiotic treatment of polyarthritis was ineffective. Many calves recovered irrespective of treatment but permanent disfigurement of the appendicular skeleton was still evident in a few calves that survived the acute stage of the disease. Isolation of the organism from mastitic quarters and bulk milk samples used to feed neonatal calves and the subsequent severe polyarthritis that coincided with the traditional prodromal period strongly implicated contaminated milk as the most likely source of infection. Mycoplasma cultures from colostral samples were unsuccessful, however only three samples were cultured and these samples may have contained sufficient antibodies against the organism to interfere with culture. Apart from the twin foetuses, which were aborted as a result of infection with *Mycoplasma* sp bovine group 7, only one other foetus was submitted for laboratory investigation during the outbreak. Mycoplasma was not isolated from this second foetus and other abortions on these properties were not investigated. Therefore it is possible that more abortions were caused by the organism. There were additional management expenses associated with the treatment of clinically affected calves and with the implementation of preventive antibiotic therapy.

There are a number of factors which may have been involved in the described outbreak on these properties. Under unified management, treatment of mastitis was similar on all three farms until the end of June 1998. It consisted of cloxacillin (Orbenin L.C. Intramammary Antibiotic Infusion; Pfizer Agricare Pty Ltd) as the first treatment for a bout of mastitis and oxytetracycline (Mastalone Blue; Pfizer Agricare Pty Ltd) as the second. Mastalone treatment was repeated when needed as a third treatment. In cases when mastitis was suspected to be caused by Gram negative organisms only Mastalone was used. In order to reduce the withholding period of milk associated with Mastalone treatment on Farm 1 and 2, Mastalone was replaced with a penicillin based antibiotic preparation (Ampiclox Lactating Cow; SmithKline Beecham Animal Health) from July 1998.

Our success in recovering *Mycoplasma* sp bovine group 7 from bulk and individual milk samples confirmed the presence of clinical and subclinical infection in the herd. Seven *Mycoplasma* isolates cultured form calves necropsied were determined to be sensitive to lincospectin, tetracycline and novobiocin but resistant to ampicillin, sulphonamide, trimethoprim, streptomycin, neomycin, erythromycin, penicillin, cloxacillin/ methicillin, lincomycin and apramycin. Since the only effective intramammary antibiotic preparation had been removed (Mastalone) it is possible that a low level of clinical and subclinical mycoplasmal mastitis, which may have existed on farms 1 and 2 could have steadily increased. By late October and early November 1998, colostral and mastitic milk contamination may have reached a critical point overriding innate resistance or passive immunity of neonatal calves, enabling the organism to cause disease. Calves were fed on mastitic milk collected from farm 1 only. Abortions may have been the result of septicaemic or mycoplasmaemic episodes in cows with clinical or subclinical mycoplasmal infection.

Mycoplasmal polyarthritis was first reported in Australia by Simons and Johnston[1] in Queensland. They described three natural cases of polyarthritis out of a herd of 27 calves and reproduced the disease by inoculating the isolated *Mycoplasma* strain into the joint cavity of a healthy calf. There was no clinical evidence of mastitis reported on the property. The *Mycoplasma* strain isolated from this outbreak was designated as PG50 and is the type strain for the *Mycoplasma* sp bovine group 7.[4]

A few years later Hughes et al[2] in NSW reported two similar outbreaks of polyarthritis on one property due to a pathogenic host-specific *Mycoplasma* sp belonging to *Mycoplasma* sp bovine group 7.[4] No sickness or mastitis had been noted in the cows and the colostrum was negative for *Mycoplasma* sp. Transmission experiments using subcutaneous and intra-conjunctival inoculations were not successful but intravenous and intra-articular injections of the organism successfully reproduced the clinical syndrome.

Mycoplasma sp isolated by Connole et al[3] in Queensland from 40 quarters of 15 cows with mastitis were serologically similar to strain PG50 and were later identified as *Mycoplasma* sp bovine group 7.[4] Infusion of cultures of the organism into one quarter of the udder of a lactating cow resulted in a clinically mild mastitis in that quarter and in the adjacent quarter on the same side. Intravenous inoculation into a calf caused polyarthritis and, although no organisms were recovered from

the joints, it was speculated that the infection had been localised in them at some stage of the disease.

Cottew [4] characterised 93 bovine strains isolated in Australia, belonging to mycoplasmas other than *M mycoides* subsp *mycoides*. Sixteen of the isolates were *Mycoplasma* sp bovine group 7, 12 of which were isolated from joints of cattle affected with arthritis including cases reported by Simons and Johnston[1] and Hughes et al.[2] Two further strains were isolated from mastitic milk[3] and two strains were obtained from lung and lymph node respectively.

In Victoria, calf polyarthritis caused by *Mycoplasma* sp bovine group 7 was first reported by Shiel et al[5] with two calves out of 10 developing the disease.

The most recent outbreak of mastitis, polyarthritis and pneumonia in calves caused by *Mycoplasma* sp bovine group 7 was reported by Alexander et al[6] from the south-west of NSW. Their investigation revealed numerous clinical cases of mycoplasmal mastitis, which were unresponsive to treatment including intramuscular tetracycline or intramammary penicillin/streptomycin combinations. Polyarthritis in reared calves was widespread affecting 10 of 22 female calves. There was no evidence of congenital infection as calves with arthritis were from clinically normal dams while the cows which developed mycoplasma mastitis delivered healthy calves.

There are significant similarities between our findings and those previously reported in Australia and overseas.[20-22] The similarities include the young age of calves affected, the great affinity of the organism for synovial epithelium, the septicaemic and fibrinopurulent nature of infection and the poor response to antimycoplasmal chemotherapy, often resulting in permanent articular disfigurement.[1-3,5,6] Mastitis as a result of *Mycoplasma* sp bovine group 7 has also been reported.[3,6] Alexander et al[6] suspected that, in their case, the most likely source of infection for young calves was contaminated colostrum or mastitic milk. Our findings also clearly indicate a link between mycoplasmal mastitis and polyarthritis in neonatal calves.

Other clinical and pathological similarities exist from observations of previous experimental infections. Intra-articular[1,2,21,22] and intravenous inoculations[2,3,20] successfully reproduced the disease in young calves but not in cattle at 12 months of age.[2,20] Attempts to infect calves via subcutaneous and supraconjunctival inoculations were unsuccessful.[2,3] Successful experimental oral transmission has not been reported but mastitis has been reproduced.[3] The lack of pathogenicity of the organism for other species in experimental inoculations, including sheep, swine, mice and guinea pigs[2,3,20] suggests some specificity of this mycoplasma for the bovine species. However the organism has been isolated from goats.[19]

Mastitis due to mycoplasma was first reported from England by Davidson and Stuart.[23] Since than it has been reported from all continents. The most common cause of mycoplasma mastitis has been *Mycoplasma bovis* and most observations and research on mycoplasma mastitis has dealt with that species.[24] In general, mycoplasma mastitis is appearing more frequently in geographic areas within and between countries. It spreads rapidly within a herd or between herds under appropriate conditions. The means of spread are not fully understood and the role of immunity or the means of establishing immunity have yet to be elucidated.[24] Economically, mycoplasmal mastitis caused by *Mycoplasma bovis* is regarded as one of the most costly infections in dairy operations.[25,26] There is no information available on the significance of *Mycoplasma* sp bovine group 7 infection in association with mastitis but epidemiological and clinical considerations may be similar to *Mycoplasma bovis*. If so, the economic losses may be substantial.

The report of the recovery of a *Mycoplasma* sp bovine group 7 from an aborted foetus was made by Langford,[9] in 1975 in North America. The isolate was from the tissues of one of 256 aborted foetuses, however the circumstances leading to abortion, the details of pathological changes and the tissues cultured were not reported. There are two other reports of bovine abortion caused by unidentified *Mycoplasma* spp. In the first report, Berry et al[13] isolated an organism with similar characteristics to mycoplasma from the abomasal content of a foetus aborted at 7 months gestation and designated the isolate as NADL 1. The dam in this case was a 44-month-old Swiss Brown cow with a history of excess lacrimation, diarrhoea and a rectal temperature of 40.6°C, one week prior to abortion. In the second report, a *Mycoplasma* strain, designated as Strain 3305 was isolated by Stalheim[10] from an aborted female Hereford foetus of about 8 months gestation. Lesions in the foetus were limited to microscopic reticuloendothelial hyperplasia in the parenchymatous organs and severe vasculitis in the blood vessels of the brain. Strain 3305 was subsequently administered intravenously to four pregnant heifers at 200 to 222 days of gestation and another three pregnant heifers at 186 to 240 days of gestation, but the experiment failed to induce abortions in any of the animals, or cause disease in dams or their calves. Page[27] also isolated a *Mycoplasma* sp from the placenta of a cow that gave birth to a calf affected by 'weak calf' syndrome. This strain was designated as Strain 3222 but later proved to be *Mycoplasma gallisepticum*[28] and was suspected to be a contaminant from embryonated eggs used during the isolation procedure.

The success in defining the aetiology of bovine abortions is reported to be low, ranging between 29.5% to 37%.[29-31] There is only one report of an abortion survey in which routine culturing for mycoplasma was attempted.[31] Of 8995 samples cultured routinely for bacteria only 794 samples were specifically cultured for mycoplasma. No mycoplasma was isolated. In many abortions, pathological changes are suggestive of infection but no infectious agent can be demonstrated.[29,31,32] It is possible, however, that some of these cases may have been caused by *Mycoplasma* spp. In our case, suspicion of mycoplasmal abortion arose only after samples collected from the aborted foetuses and placed on blood and MacConkey agar plates were fortuitously incubated for up to 7 days. The beta-haemolytic colonies appeared 5 days after incubation and did not stain using the Gram method, strongly suggesting the presence of *Mycoplasma* sp. If the culture plates had been incubated for a shorter period as is usually the case, the diagnosis may have been missed.

Much has to be learnt about the role and significance of *Mycoplasma* sp bovine group 7 in causing disease in cattle. There is a general lack of information on it's potential involvement in mastitis[24] and other diseases.[30,33,34] Nevertheless our findings indicate that *Mycoplasma* sp bovine group 7 can, under certain circumstances, be an important pathogen, capable of causing a variety of clinical syndromes with subsequent significant economic losses. Traditionally, diagnosis of mycoplasmosis is difficult, mainly because it requires special culture procedures which are usually not available in routine veterinary diagnostic laboratories. It is likely therefore that diseases caused by mycoplasmas are often missed. Furthermore, since clinical and pathological findings caused by infection with mycoplasmas are essentially similar to other bacterial infections, diseases

primarily caused by mycoplasmas may be attributed to other bacteria if specific mycoplasma culture is not performed. These other bacteria may be secondary invaders or present as contaminants in the clinical sample. Further research is needed to define the presence of *Mycoplasma* sp bovine group 7 infection in dairy herds and to ascertain it's economic significance.

Acknowledgments

Financial support associated with this investigation was provided by NSW Agriculture's Elizabeth Macarthur Agricultural Institute, Research and Development Allocation. We thank NSW Agriculture staff pathologists for their critical comments and helpful suggestions throughout this investigation. The figures were prepared by Mr Lowan Turton.

References

1. Simmons GC, Johnston LAY. Arthritis in calves caused by *Mycoplasma* sp. *Aust Vet J* 1963;39:11-14.
2. Hughes KL, Edwards MJ, Hartley WJ, Murhy S. Polyarthritis in calves caused by *Mycoplasma sp. Vet Rec* 1966;78:276-280.
3. Connole MD, Laws L, Hart RK. Mastitis in cattle caused by a *Mycoplasma* sp. *Aust Vet J* 1967;43:157-162.
4. Cottew GS. Mycoplasmas isolated from cattle in Australia. *Aust Vet J* 1970;46:378-381.
5. Shiel MJ, Coloe PJ, Worotniuk B, Burgess GW, Polyarthritis in calf associated with a group 7 mycoplasma infection. *Aust Vet J* 1982;59 192-193.
6. Alexander PG, Slee KJ, McOrist S, Ireland L, Coloe PJ. Mastitis in cows and polyarthritis and pneumonia in calves caused by *Mycoplasma* species bovine group 7. *Aust Vet J* 1985;62: 135-136.
7. Edward DG, Hancock JL, Highnett SL. Isolation of pleuropneumonia-like organism from the bovine genital tract. *Vet Rec* 1947;59:329-330.
8. Stone SS, Masiga WN, Read WCS. *Mycoplasma mycoides* transplacental transfer in cattle. *Res Vet Sci* 1969;10:368-372.
9. Langford EV. Mycoplasma recovered from bovine male and female genitalia and aborted foeti. *Annu Proc Am Assoc Vet Lab Diagnosticians* 1975;18:221-219.
10. Stalheim OHV, Hubbert WT, Foley JW. Infectivity of two mycoplasmas of bovine origin in pregnant heifers. *Am J Vet Res* 1974;35:63-66.
11. Byrne WJ, Brennan P, McCormack R, Ball HJ. Isolation of *Mycoplasma bovis* from the abomasal contents of an aborted bovine fetus. *Vet Rec* 1999;144:211-212.
12. Romvary J, Rozsa J, Stipkovits L, Szabo J. Arthritis and pneumonia caused by mycoplasma in cattle. *Magyar Allatorvosok Lapja* 1975;30:695-698.
13. Berry PA, Bryner JH, Frank AH. Isolation of mycoplasma from an aborted bovine fetus and vaginal mucus. *Am J vet Res* 1966;27:677-681.
14. Chappel RJ. Leptospirosis. In:Corner LA, Bagust TJ, editors, *Australian Standard Diagnostic Techniques for Animal diseases,* CSIRO for the Standing Committee on Agriculture and Resource Management, East Melbourne, 1993.
15. Kirkland PD, MacKintosh SG. Bovine Pestivirus Infection. In:Corner LA, Bagust TJ, editors. *Australian Standard Diagnostic Techniques for Animal diseases.* CSIRO East Melbourne, 1993.
16. Hovind-Hougen K, Friis NF. Morphological and ultrastructural studies of *Mycoplasma flocculare* and *Mycoplasma hyopneumoniae* in vitro. *Res Vet Sci* 1991;51:155-163.
17. Tully JG (1983). Cloning and filtration techniques for mycoplasmas. In:Razin S, Tully JC, editors. *Methods in Mycoplasmology* Vol 1. Academic Press, New York, 1983:173-177.
18. Clyde, W.A . Mycoplasma species identification based on growth inhibition by specific antisera. *J Immunol* 1964;92:958-965.
19. Frey J, Cheng X, Moncrat MP et al. Genetic and serologic analysis of the immunogenic 67-kDa lipoprotein of mycoplasma sp. bovine Group 7. *Res Microbiol* 1998;149:55-64.
20. Moulton JE, Boidin AG, Rhode EA. A pathogenic pleuropneumonia-like organism from a calf. *J Am Vet Med Ass* 1956;129:364-367.
21. Corboz L, Keller A, Waldvogel A, Weideli U. Über Spontan- und Experimentalfälle von Polyarthritis und -synovitis bei Kälbern, verursacht durch Mycoplasmen II. *Schweiz Arch Tierheilk* 1980;122:479-491.
22. Keller H, Corboz L, Waldvogel A, Weideli U. Über Spontan- und Experimentalfälle von Polyarthritis und -synovitis bei Kälbern, verursacht durch Mycoplasmen I. Klinishe Aspecte. *Schweiz Arch Tierheilk* 1980;122:15-26.
23. Davidson I, Stuart P. Isolation of a *mycoplasma*-like organism from an outbreak of bovine mastitis [letter]. *Vet Rec* 1960;72:766.
24. Jasper DE. Bovine mycoplasma mastitis. *Adv Vet Sci Comp Med* 1981;25:121-159.
25. Ebenhart RJ, Harmon RJ, Jasper DE et al. *Current concepts of bovine mastitis*, 3rd edn. National Mastitis Council, Arlington, VA 1987.
26. Ghadersohi A, Hirst RG, Forbes-Faulkner J, Coelen RJ. Preliminary studies on the prevalence of *Mycoplasma bovis* mastitis in dairy cattle in Australia. *Vet Microbiol* 1999;65:185-194.
27. Page LA, Frey ML, Ward K et al. Isolation of a new serotype of mycoplasma from a bovine placenta *J Am Vet Med Assoc* 1972;161:919-925
28. Rhoades KR, Page LA. Identification of bovine mycoplasma strain 3222 as *M gallisepticum. Vet Rec* 1975;96:460.
29. Jerrett IV, McOrist S, Wadington J et al. Diagnostic studies of the fetus, placenta and maternal blood from 265 bovine abortions, *Cornell Vet* 1984;74:8-20.
30. Murray RD. A field investigation of causes of abortion in dairy cattle. *Vet Rec* 1990;127:543-547.
31. Kirkbride CA. Etiologic agents detected in a 10-year study of bovine abortions and stillbirths *J Vet Diagn Invest* 1992;4:175-180.
32. David A, McEven B, Hazlett M, Maxie G, Anderson N. Trends in bovine abortions submitted to the Ontario Ministry of Agriculture, Food and Rural Affairs, 1993-1995. *Can Vet J* 1996;37:287-288.
33. Langford EV. Mycoplasma species recovered from the reproductive tracts of western Canadian cows. *Can J comp Med* 1975;39:133-138.
34. Stalheim OHV. Laboratory diagnosis in food animals. *Special report of the Mycoplasmosis Committee,* American Association of Veterinary Laboratory Diagnosticians, Madison, Wisconsin, 1996.

(Accepted for publication 1 May 2000)

CORRECTION

Eimeria dunsingi in free living musk lorikeets (*Glossopsitta concinna)* by Gartrell BD, O'Donoghue P and Raidal SR. *Aust Vet J* 2000;78:717-718.

Due to a printing error, the above article was not included at the end of the October issue of the *Australian Veterinary Journal*, though it appeared in the contents page. A copy of the page containing the article has been printed and is included as an insert with the November Journal. This page can be added to the October pages when binding. The October Journal that appears on the AVA website is complete.