JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1975, p. 165-168
Copyright © 1975 American Society for Microbiology
Vol. 2, No. 3
Printed in U.S.A.

Naturally Occurring and Experimentally Induced Mycoplasmal Arthritis of Cattle

O. H. V. STALHEIM* AND L. A. PAGE

National Animal Disease Center, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received for publication 7 April 1975

Mycoplasma agalactiae subsp. *bovis* strain Iowa 1136 was isolated from synovial fluids of a clinical case of arthritis in cattle on pasture in Iowa. When given to calves and cows by intra-articular or intravenous injection, it caused severe and persistent joint infections with fever, lameness, and swelling of the affected joints, plus synovitis, tendonitis, and fibrinous-purulent synovial fluids of high protein content. Intramammary administration of the organism caused severe mastitis. Calves nursing the cows developed severe mycoplasmal arthritis.

In 1956, Moulton et al. (13) reported the first case of mycoplasmal arthritis in cattle since the eradication of bovine pleuropneumonia from this country in 1892. Intravenous administration of the mycoplasma to calves caused fever, severe arthritis, and keratitis. At necropsy, the organism was recovered from the synovial fluids, liver, and spleen. The culture was recently reported as antigenically distinct from all other mycoplasmas of bovine origin (1). Similar reports of bovine mycoplasmal arthritis in association with pulmonary disease appeared in England (8) and Australia (15). The Australian isolate was recently identified as a distinct mycoplasma species (3, 11).

During a severe outbreak of mastitis in California in 1966, mycoplasmas were isolated from the joint fluids and milk of two arthritic dairy cows (9). They were identified as *Mycoplasma bovimastitidis*, which is now named *M. agalactiae* subsp. *bovis* (11). This same organism was recently isolated from joint fluids of arthritic feedlot cattle in California (7) and in Canada (16). This report concerns clinical aspects of an outbreak of mycoplasmal arthritis in Iowa cattle on pasture and the reproduction of the disease in calves and adult cattle. Procedures for the identification of the isolate as *M. agalactiae* subsp. *bovis* are reported separately (18).

MATERIALS AND METHODS

Specimens. Twenty Angus-Hereford heifers were purchased at a salesbarn and placed on pasture in Iowa. Approximately 60 days later, eight were lame with swollen carpal joints. By aspiration, 10 ml of viscous, turbid, whitish fluid was collected from the carpal joint of a heifer that was lame. It contained a mycoplasma that was designated strain Iowa 1136 and classified as *M. agalactiae* subsp. *bovis* (18).

Experimentally induced bovine mycoplasmal arthritis. Twelve cows and seven calves from the National Animal Disease Center herd were placed in isolation and examined for mycoplasma. Swabs of nasal and vaginal mucus were cultured in mycoplasma medium (10). The isolates were characterized biochemically and identified by serology (17). None were *M. agalactiae* subsp. *bovis*. After artificial exposure by different routes to strain Iowa 1136 (Table 1), the cattle were observed daily, rectal temperatures were recorded, and blood samples were cultured for mycoplasmas. When lameness was observed, a physical examination was made of the cow or calf, and a sample of synovia was obtained from the affected joint. It was inoculated on plates of mycoplasma medium (10) and decimally diluted in tubes of broth to determine the approximate number of mycoplasmas per milliliter of fluid. Samples of blood and secretions were similarly cultured and titrated. Fecal material on cotton swabs was placed in mycoplasma medium and shaken, a sample was centrifuged at low speed, and the supernatant fluid was forced through a cellulose filter pad (0.45-μm pores) onto a plate of agar medium. Mycoplasma isolates were characterized by biochemical tests and identified with antiserum to strain Iowa 1136 by growth inhibition or fluorescent antibody tests (18, 20).

After observations for 14 to 28 days, the cattle were killed by injections of succinylcholine chloride and necropsied. Blood, urine, and pericardial fluids were cultured for mycoplasma, along with samples of synovial fluids from several diarthroses: allantoaxial, scapulohumeral, radiohumeral, and the carpal-metacarpal sac of the carpus, as well as the ischiofemoral, phallangeo-metacarpal, and femorotibiae. Parts of the capsule of affected joints were removed, minced, homogenized in a blender, and decimally diluted in mycoplasma medium. Other affected parts were fixed in 10% formalin solution and sectioned. The sections were stained with hematoxylin and eosin and studied microscopically.

TABLE 1. *Route of administration and dose in experimental bovine mycoplasmosis*

Expt no.	Route of inoculation	No. of cattle	No. of organisms
1	Intra-articular	5	2×10^9
		7 (calves)	2×10^9
2	Intravenous	5	2×10^{10}
3	Intramammary	2	9×10^9

Intra-articular administration to cows and calves (experiment no. 1). One milliliter of a 48- to 72-h culture of strain Iowa 1136 was injected into the carpal-metacarpal sac of eight carpal joints of five Holstein cows and into both carpal joints of seven 1- to 3-month-old Holstein calves. After the hair was clipped and a germicide applied, a sterile, 16-guage needle was introduced into the synovial sac, and a sample of synovia was removed. The culture was then injected through the same needle. During the period of observation, samples of synovial fluid were similarly collected. Heparin was added to fluid from infected joints to prevent coagulation. Samples of synovia were dried on glass slides, stained with Giemsa stain, and examined microscopically. The protein content was determined by refractometry.

Intravenous administration to cows (experiment no. 2). Ten milliliters of a culture of strain Iowa 1136 was injected into the blood of the jugular vein of five Angus-Holstein cows. Three of the cows were pregnant (third trimester of gestation).

Intramammary administration to cows (experiment no. 3). After a sample of milk was collected for culture and a germicide was applied to the end of the teat, 3 ml of strain Iowa 1136 was injected into each of the right quarters of the udders of two lactating Holstein cows. Each cow was nursing a 48-h-old calf. Samples of milk were collected daily from each quarter, cultured for mycoplasmas, and tested for evidence of mastitis by the California mastitis test.

RESULTS

Experimental bovine arthritis. Tests of cows and calves for mycoplasmas before artificial exposure revealed that three cows and three calves harbored mycoplasmas. One calf was a nasal carrier of *M. bovirhinis*; five other isolates were non-sterol requiring (acholeplasmas). After intra-articular administration of *M. agalactiae* subsp. *bovis* strain Iowa 1136 into eight carpal joints of five cows, all the cows developed fever, severe lameness, and swelling of the exposed joints. The fever was detected in 24 h; it usually reached a peak of 40.0 to 41.0 C on day 2 or 3 postinfection (p.i.) and then declined to 39.5 ± 0.5 C for the rest of the observation period. The lameness developed 12 to 24 h after exposure and persisted throughout the observation period. Four of the five cows became very lame from day 7 to 14 p.i. They remained recumbent most of the time and arose only with difficulty. Only the inoculated joints appeared to be affected. All the cows had partial anorexia and lost weight; two cows lost an estimated 100 kg.

Intra-articular administration to cows and calves. Strain Iowa 1136 was recovered one or more times from synovial fluids of each exposed carpal joint. Mycoplasemia was detected sporadically in all five cows; the results of cultural examination of cow no. 2 are summarized (Table 2). The organism persisted in affected joint fluids for 28 days, the longest time tested.

At necropsy, strain Iowa 1136 was recovered from 7 of 8 exposed carpal joints. Although the left carpal joint of cow no. 5 contained $>10^8$ mycoplasmas/ml on day 8, cultures were negative at necropsy on day 14. In addition, the organism was recovered from synovial fluids of the elbow (cows no. 1 and 3; $>10^4$/ml), the shoulder joint (cow no. 4; $>10^4$/ml), the vagina (cow no. 4), and the right nostril (cow no. 3).

When placed in the carpal joints of seven calves, strain Iowa 1136 caused arthritis similar to the disease in cows, but the lameness and swelling of affected joints were more severe. Three calves developed ulcers on the anterior surface of the knees; these ulcers progressed to rupture of the distended synovial sac and drainage of yellowish, thick fluids. During the experiment the organism was recovered from all 14 exposed joints in high titers ($>10^8$/ml). At necropsy, all 14 inoculated joints yielded the organism. In addition, *M. agalactiae* subsp. *bovis* was recovered from the conjunctival sac and

TABLE 2. *Recovery of M. agalactiae subsp. bovis during experimental bovine mycoplasmal arthritis (cow no. 2; intra-articular inoculation)*[a]

Days post-exposure	Mycoplasma recovered from:			
	Blood (mycoplasmas/ml)	Carpal joint (mycoplasmas/ml)	Urine	Nose
0	–	–	–	–
1	–	$+(2 \times 10^2)$	–	–
2	–	$+(4 \times 10^2)$	–	–
5	–	$+(4 \times 10^8)$	–	–
8	–	+	–	–
9	–	ND[b]	ND	ND
13	$+(2 \times 10^4)$	$+(2 \times 10^6)$	–	–
15	+	+	–	–
17	ND	$+(2 \times 10^4)$	–	–
21	$+(10^4)$	$+(2 \times 10^5)$	–	ND
26	–	+	–	–
28	–	$+(4 \times 10^4)$	–	–

[a] After intra-articular administration into the left carpal joint, cultural examinations were made for 28 days, when the cow was killed.

[b] ND, Not done.

urine of calf no. 7. The infected eye had a normal appearance.

Intravenous administration to cows. By this route, *M. agalactiae* subsp. *bovis* strain Iowa 1136 caused cellulitis at the site of administration, moderate fever, and arthritis. Three of the five cows developed diffuse, localized reactions on the neck involving the skin and subcutaneous tissues within 6 cm of the site of administration. The cellulitis was first detected on day 4 p.i.; it continued for 5 to 7 days and then resolved without treatment. Three cows had fevers that reached 39.5 C; one cow had a fever of 40.2 C for 2 days. Other clinical signs were moderate inappetence and lassitude from days 2 to 4 p.i.

On day 15, cow no. 10 became lame on the right front leg. Tenderness of the elbow joint was detected by palpation; the joint was not swelled and was not warm to the touch. The lameness increased on day 16, and the cow became recumbent most of the time until necropsy on day 27. The rectal temperature varied from 38.5 to 39.5 C. At necropsy, the right elbow contained thin, nonpurulent, yellowish fluid; *M. agalactiae* subsp. *bovis* was recovered ($>10^8$/ml). Lesions of arthritis and tendonitis were observed. Results from all other cultural attempts of other joints and organs, of the fetal (25 kg) blood, liver and joints, or of amniotic fluid were negative, except *M. agalactiae* subsp. *bovis* was recovered from a cotyledon.

On day 18, cow no. 6 gave birth to a normal bull calf that weighed 42.7 kg. On day 20, cow no. 6 and her calf were killed, along with cows no. 7, 8, and 9. Cow no. 7 was pregnant with a fetus weighing 29 kg. Strain Iowa 1136 was not recovered from the four cows, the fetus, or the calf.

Intramammary administration to cows. On day 1 p.i., cows no. 11 and 12 were feverish (39.8 C) and anorexic. The right quarters of the udders of both cows were slightly swollen. The watery, lacteal secretions contained a few clots or flakes and gave +3 reactions with the California mastitis test. Mycoplasmas were isolated ($>10^6$ organisms/ml). The swelling and firmness of the right quarters continued and progressed to a maximum on days 4 to 6 and then decreased slightly until necropsy on day 13. The amount of secretion decreased by approximately 90%; it became thick and purulent and continued to cause +3–4 reactions with the California mastitis test. Titers of mycoplasmas in the right quarters ranged from 10^8 to 10^{10}/ml. On day 8 p.i., *M. agalactiae* subsp. *bovis* was isolated from the uninoculated (left) quarters of both cows. Clinical mastitis was observed in the left quarters the next day; the course was similar to that of the right quarters.

On day 8 p.i., the calf that was nursing cow no. 11 became lame, and both knees were slightly swollen. A sample of synovial fluid contained *M. agalactiae* subsp. *bovis* ($>10^2$/ml). On day 9, the other calf was also lame. The next day, both knees were swollen and *M. agalactiae* subsp. *bovis* was isolated from samples of synovial fluid. On day 12, both calves were very lame; *M. agalactiae* subsp. *bovis* was isolated from all eight quarters of the cows (10^8 to 10^{10}/ml), from both carpal joints of each calf ($>10^2$/ml), and from the nasal mucus of cow no. 11 and of the calf of cow no. 12.

At necropsy on day 16, *M. agalactiae* subsp. *bovis* was isolated from blood, nasal mucus, and synovial fluids of the carpal, elbow, and hock joints of both calves and from the udder, uterus, and vagina of both cows. Urine was not cultured; fecal isolates proved to be acholeplasmas.

Synovial fluids from normal (nonexposed) cows contained 4 to 7 mg of protein per ml. Twenty days after intra-articular administration of *M. agalactiae* subsp. *bovis*, synovial fluid from affected joints of two cows contained an average of 45.4 mg of protein per ml (range of 44 to 47).

Pathology. Intra-articular administration of *M. agalactiae* subsp. *bovis* strain Iowa 1136 induced a severe fibrino-purulent synovitis that progressed to hypertrophy, clot formation, and consolidation. Macroscopic observation of the synoviae revealed villous outgrowths, petechial hemorrhages, erythema, and small nodules of fibrin. Consolidation of the fibrinous exudate sometimes led to the formation of large, sequestered clots, 3 to 5 cm long. Microscopically, the clots consisted of compressed fibrin and degenerating neutrophiles. Sections of the joint capsule showed a fibrinous arthritis with proliferation of synovial cells and the formation of many villi. The tendon sheaths of the extensor tendons crossing the carpal joint were extensively involved with pathologic changes similar to those of the soft tissues of the joint.

DISCUSSION

M. agalactiae subsp. *bovis* (formerly named *M. bovimastitidis*) is a versatile pathogen. It caused cytopathic effects in cell cultures (6), mastitis, vaginitis, and salpingo-oophoritis in the cow (4, 5, 9; P. A. O'Berry, Ph.D. thesis, Iowa State University, Ames, 1967) and seminal vesiculitis in the bull (personal communication, D. Lein and M. Tourtellotte), as well as pneumonia (14) and intractable arthritis, sometimes with signs and lesions of respiratory disease (8, 15). In the present study, strain 1136, an isolate from arthritic Iowa feeder cattle,

caused severe arthritis when given by intravenous or intra-articular injections and persisted in affected joints 28 days, the longest time tested. Other strains isolated from arthritic feeder cattle in Nebraska also caused bovine arthritis under experimental conditions (personal communication, E. L. Stair, Jr.). The initial inflammatory response to mycoplasmal arthritis is both purulent and fibrinous in character. Later the reaction is primarily fibrinous, with increased proteins in the contents of the joint capsule. Resolution of the exudate allows for gradual restoration of function.

During severe outbreaks of calf pneumonia or "shipping fever" of older cattle, *M. agalactiae* subsp. *bovis* was isolated at necropsy from pneumonic lungs and from the nasal mucus of convalescent cattle; other recognized agents of bovine respiratory disease were not recovered (unpublished data). During the present experiments, *M. agalactiae* subsp. *bovis* was recovered from the placenta, uterus, urine, and nasal and vaginal mucus of exposed cattle and transmitted to calves via infective milk.

Based on these clinical observations and experimental studies, the following hypothesis of transmission and pathogenesis is advanced. By close contact during transportation or rearing, vaginal or nasal carriers of *M. agalactiae* subsp. *bovis* provide exposures to susceptible cattle, which under stressful conditions (changes in feed, weather, etc.) may progress to clinical cases of pneumonia or arthritis. During hematogenous dissemination, the organisms lodge in the smaller bronchioles and capillaries of the lungs or the rete carpi dorsale, the network of fine vessels surrounding the carpal joint, or both. By extension, the plastic organisms reach the synovial membrane and fluids of the carpal joint and multiply and thus cause arthritis. They may persist in synovial fluids for a month, with sporadic episodes of mycoplasemia (Table 2) and dissemination. Therefore, the transmission of *M. agalactiae* subsp. *bovis* among cattle is not unlike that of *M. mycoides* var. *mycoides*, the cause of contagious bovine pleuropneumonia, i.e., via infective droplets of respiratory secretions or urine (12) and perhaps in utero to the fetus (19). In these experiments *M. agalactiae* subsp. *bovis* was recovered from the placenta but not from the fetuses. Further studies are necessary on its role in fetal death and abortion.

LITERATURE CITED

1. Al-Aubaidi, J. M., and J. Fabricant. 1971. Characterization and classification of bovine mycoplasma. Cornell Vet. 61:490–518.
2. Cordy, D. R. 1959. Arthritis in ruminants due to pleuropneumonia-like organisms. Lab. Invest. 8:1432–1434.
3. Cottew, G. S. 1970. Mycoplasmas isolated from cattle in Australia. Aust. Vet. J. 46:378–381.
4. Hale, H. H., C. F. Helmboldt, W. N. Plastridge, and E. F. Stula. 1962. Bovine mastitis caused by a *Mycoplasma* species. Cornell Vet. 52:582–591.
5. Hirth, R. S., S. W. Nielsen, and W. N. Plastridge. 1966. Bovine salpingo-oophoritis produced with semen containing a *Mycoplasma*. Pathol. Vet. 3:616–632.
6. Hirth, R. S., M. E. Tourtellotte, and S. W. Nielsen. 1970. Cytopathic effects and ultrastructure of *Mycoplasma agalactiae* var. *bovis* (Donetta strain). Infect. Immun. 2:105–111.
7. Hjerpe, C. A., and H. D. Knight. 1972. Polyarthritis and synovitis associated with *Mycoplasma bovimastitidis* in feedlot cattle. J. Am. Vet. Med. Assoc. 160:1414–1418.
8. Hughes, K. L., M. J. Edwards, W. J. Hartley, and S. Murphy. 1966. Polyarthritis in calves caused by *Mycoplasma sp.* Vet. Rec. 78:276–280.
9. Jasper, D. E., N. C. Jain, and L. H. Brazil. 1966. Clinical and laboratory observations on bovine mastitis due to *Mycoplasma*. J. Am. Vet. Med. Assoc. 148:1017–1029.
10. Kelton, W. H., and H. Van Roekel. 1963. Serological studies of mycoplasma (PPLO) of avian origin. Avian Dis. 3:272–286.
11. Leach, R. H. 1973. Further studies on classification of bovine strains of mycoplasmatales, with proposals for new species, *Acholeplasma modicum* and *Mycoplasma alkalescens*. J. Gen. Microbiol. 75:135–153.
12. Masiga, W. N., R. S. Windson, and W. C. S. Read. 1972. A new mode of spread of contagious bovine pleuropneumonia? Vet. Rec. 90:247–248.
13. Moulton, J. E., A. G. Boidin, and E. A. Rhode. 1956. A pathogenic pleuropneumonia-like organism from a calf. J. Am. Vet. Med. Assoc. 129:364–367.
14. Onoviran, O. 1972. Experimental infection of the respiratory tract of zebu cattle with *Mycoplasma agalactiae* varias *bovis*. Bull. Epizoot. Dis. Afr. 20:275–279.
15. Simmons, G. C., and L. A. Y. Johnston. 1963. Arthritis in calves caused by *Mycoplasma* sp. Aust. Vet. J. 39:11–14.
16. Singh, U. M., P. A. Doig, and H. L. Ruhnke. 1971. Mycoplasma arthritis in calves. Can. Vet. J. 12:183–185.
17. Stalheim, O. H. V., W. T. Hubbert, and J. W. Foley. 1974. Infectivity of two mycoplasmas of bovine origin in pregnant heifers. Am. J. Vet. Res. 35:63–66.
18. Stalheim, O. H. V., and S. S. Stone. Isolation and identification of *Mycoplasma agalactiae* subsp. *bovis* from arthritic cattle in Iowa and Nebraska. J. Clin. Microbiol. 2:169–172.
19. Stone, S. S., W. N. Masiga, and W. C. S. Read. 1969. *Mycoplasma mycoides* transplacental transfer in cattle. Res. Vet. Sci. 10:368–372.
20. Stone, S. S., and J. Tessler. 1974. Fluorescent labeling of antibody for identifying mycoplasma colonies by incident ultraviolet light. Am. J. Vet. Res. 35:107–110.